ISSN 0022-4847
DOI:10.6156/JTAR

台灣農業研究

JOURNAL OF TAIWAN AGRICULTURAL RESEARCH

Vol.67 No.4 2018

研究報告

323 台灣寬薊馬亞科 (纓翅目：管尾亞目：管尾薊馬科) 之分類描述I雙毛族
王清玲、陳怡如、林鳳琪

337 瓜實蠅 [*Bactrocera* (*Zeugodacus*) *cucurbitae* (Coquillett)] 中胸背板剛毛遺傳變異之縮減
鄭允、王志賢、黃毓斌、江明耀、呂秀英、吳東鴻、楊棠民、粘君琪

347 番椒微斑病毒 (*Pepper mild mottle virus*) 田間發生與器械消毒管理
曹麗玉

355 香菇柄水萃物改善糖尿病降低阿茲海默氏症罹病風險初探
楊淑惠、楊惠婷、蔣淑琦

365 評估氯化鐵清洗法對含鎘水稻田土壤鎘移除、土壤性質及稻作生長之影響
許健輝、林毓雯、鄭佳怡、何雅婷、陳柱中、劉滄棽、郭鴻裕

377 利用中和亞磷酸溶液防治馬鈴薯青枯病
林靜宜、林慧如

387 柑橘褐斑病之病原特性及防治藥劑篩選
黃巧雯、吳昭蓉、楊宏仁、賴素玉、倪蕙芳

401 植物生長調節劑與兩階段培養對薑組織培養苗增殖與生長之影響
陳威臣、曹進義、吳姿穎、夏奇鈮

行政院農業委員會農業試驗所出版

台灣農業研究
JOURNAL OF TAIWAN AGRICULTURAL RESEARCH
Vol.67 No.4 2018

台灣農業研究

JOURNAL OF TAIWAN AGRICULTURAL RESEARCH

Vol.67 No.4 2018

Research Articles

323 Taxonomic Description of Idolothripinae (Thysanoptera: Tubulifera: Phlaeothripidae) of Taiwan. I. Tribe Idolothripini
Chin-Ling Wang, Yi-Ju Chen, and Feng-Chyi Lin

337 Reduction in Genetic Variability for Quantitative Trait Prescutellar Bristle Number of Melon Fly [*Bactrocera* (*Zeugodacus*) *cucurbitae* (Coquillett)]
Edward Yun Cheng, Zu-Hsien Wang, Yu-Bing Huang, Ming-Yaw Chiang, Hsiu-Ying Lu, Dong-Hong Wu, Chung-Min Yang, and Chun-Chi Nien

347 Investigation of *Pepper mild mottle virus* on Peppers and the Disinfection of Cutting Tools in Taiwan
Li-Yu Tsao

355 Preliminary Study of *Lentinus edodes* Stipe Extracts on Reducing the Risk of Alzheimer's Disease in Diabetic Animal Model
Shu-Hui Yang, Hui-Ting Yang, and Shu-Chi Chiang

365 Assessing the Effects of Soil Washing with Ferric Chloride on Cadmium Removal, Soil Properties and Rice Growth in Cd-containing Paddy Fields
Chien-Hui Syu, Yu-Wen Lin, Chia-Yi Cheng, Ya-Ting Ho, Chu-Chung Chen, Tsang-Sen Liu, and Horng-Yuh Guo

377 Control of Bacterial Wilt of Potato with Neutralized Phosphorous Acid
Ching-Yi Lin and Hui-Ju Lin

387 Physiological Characteristics, Pathogenicity and Fungicide Screening of Citrus Alternaria Brown Spot Disease Caused by *Alternaria alternata*
Chiao-Wen Huang, Chao-Jung Wu, Hong-Ren Yang, Su-Yu Lai, and Hui-Fang Ni

401 Effect of Plant Growth Regulator and Two-Stage Culture on Proliferation and Growth of *In Vitro* Ginger Plantlets
Uei-Chern Chen, Chin-Yi Tsao, Tzu-Ying Wu, and Chi-Ni Hsia

ISSN 0022-4847
9 770022 484003

Published by Taiwan Agricultural Research Institute, Council of Agriculture

《台灣農業研究》編審會

總編輯

楊純明／農業試驗所作物組

領域主編 (按筆劃序)

王強生／國立中興大學農藝學系
王淑珍／國立台灣大學農藝學系
古新梅／國立中興大學農藝學系
安寶貞／農業試驗所植物病理組
吳文哲／國立台灣大學昆蟲學系
呂秀英／苗栗區農業改良場
杜宜殷／國立台灣大學園藝暨景觀學系
沈偉強／國立台灣大學植物病理與微生物學系
林彥蓉／國立台灣大學農藝學系
林慧玲／國立中興大學園藝學系
侯豐男／國立中興大學昆蟲學系
柯俊成／國立台灣大學昆蟲學系
高景輝／國立台灣大學農藝學系
張永和／靜宜大學食品營養系
張瑞璋／農業試驗所植物病理組
莊愷瑋／國立嘉義大學農藝學系
郭寶錚／國立中興大學農藝學系
陳世銘／國立台灣大學生物產業機電工程學系
陳右人／國立台灣大學園藝暨景觀學系
陳宗禮／國立中興大學農藝學系
陳郁蕙／國立台灣大學農業經濟學系
陳健忠／農業試驗所應用動物組
陳琦玲／農業試驗所農業化學組
曾夢蛟／國立中興大學園藝學系
黃振文／國立中興大學植物病理學系
黃裕銘／國立中興大學土壤環境科學系
黃肇家／農業試驗所作物組
黃鵬林／國立台灣大學園藝暨景觀學系
楊正澤／國立中興大學昆蟲學系
路光暉／國立中興大學昆蟲學系
鄒裕民／國立中興大學土壤環境科學系
劉力瑜／國立台灣大學農藝學系
蔡致榮／農業試驗所
鍾文鑫／國立中興大學植物病理學系
簡立賢／國立中興大學應用經濟學系
顏國欽／國立中興大學食品暨應用生物科技學系

編輯助理

吳惠卿／農業試驗所技術服務組
王紫瑜／農業試驗所《台灣農業研究》期刊編輯室
王立蓉／農業試驗所《台灣農業研究》期刊編輯室

編輯顧問

吳宜晏／農業試驗所技術服務組

台灣農業研究

第六十七卷　第四期　　　　中華民國一〇七年十二月三十一日

出版者　行政院農業委員會農業試驗所
發行人　陳駿季
總編輯　楊純明
地址　41362 台中市霧峰區萬豐里中正路 189 號
網址　http://www.tari.gov.tw
電話　(04)2331-7236
E-mail　jtar@tari.gov.tw
線上投審稿系統　http://www.ipress.tw/J0042

編印　Ainosco Press
地址　23452 新北市永和區成功路一段 80 號 18 樓
電話　(02)2926-6006
E-mail　press@airiti.com

定價　新台幣一七五元
展售門市　1. 國家書店 / 10485 台北市松江路 209 號 1 樓 / 電話 : (02) 2518-0207
國家網路書店 http://www.govbooks.com.tw
2. 五南文化廣場 / 40042 台中市中山路 6 號 / 電話 : (04) 2226-0330

台灣農業研究 (J. Taiwan Agric. Res.) 67(4):323–336 (2018)
DOI:10.6156/JTAR.201812_67(4).0001

Research Article

Taxonomic Description of Idolothripinae (Thysanoptera: Tubulifera: Phlaeothripidae) of Taiwan. I. Tribe Idolothripini

Chin-Ling Wang[1], Yi-Ju Chen[2], and Feng-Chyi Lin[3,*]

Abstract

Wang, C. L., Y. J. Chen, and F. C. Lin. 2018. Taxonomic description of Idolothripinae (Thysanoptera: Tubulifera: Phlaeothripidae) of Taiwan. I. Tribe Idolothripini. J. Taiwan Agric. Res. 67(4):323–336.

Five genera and 11 species of Tribe Idolothripini of subfamily Idolothripinae in Taiwan are reviewed. Identification keys are provided, and diagnostic characteristics are described and illustrated. *Dinothrips spinosus* (Schmutz) is the first species and genus recorded in Taiwan.

Key words: Taxonomy, Thysanoptera, Phlaeothripidae, Idolothripinae, Taiwan.

INTRODUCTION

The thrips, insects belonging to order Thysanoptera, are divided into two suborders, Terebrantia and Tubulifera. The suborder Tubulifera contains a single family Phlaeothripidae which is divided into two subfamilies, Idolothripinae and Phlaeothripinae. In this study, the members of Idolothripini of subfamily Idolothripinae in Taiwan are reviewed.

More than 80 genera and 700 species of Idolothripinae have been described worldwide (ThripsWiki 2018). The world genera of Idolothripinae was reviewed by Mound & Palmer (1983). Eow *et al*. (2011) provided a key to 31 genera and 34 species of Idolothripinae in Peninsular Malaysia and Singapore. Majid *et al*. (2011) listed 59 species and 18 genera of this subfamily in China; the number increased to 62 species and 19 genera by Dang & Qiao (2013) who provided illustrated keys to these taxa.

For Taiwanese Idolothripini, Karny (1913b) first described 2 new genera and species: *Phoxothrips pugilator* (= *Mecynothrips pugilator*) and *Idolothrips formosanus* (= *Ophthalmothrips formosanus*). Priesner (1935a) published three new species, *Elaphrothrips mucronatus*, *E. sumbanus*, and *E. productus*, though all species are synonyms of *E. denticollis* Bagnall (Mound & Palmer 1983). Priesner (1935a) also listed *E. formosanus* (Karny) [= *Ophthalmothrips formosanus* (Karny)]. Priesner (1935b) described two new species, *Elaphrothrips clarispinis* (= *E. spiniceps* Bagnall) and *E. takahashii* [= *Mecynothrips pugilator* (Karny)]. Forty years later, Haga (1975) recorded *Pyrgothrips formosanus* (Karny) [= *Ophthalmothrips formosanus*

Received: March 4, 2018; Accepted: May 13, 2018.
* Corresponding author, e-mail: fclin@tari.gov.tw
1 Former Research Fellow, Applied Zoology Division, Taiwan Agricultural Research Institute, Council of Agriculture, Taichung, Taiwan, ROC.
2 Assistant Research Fellow, Applied Zoology Division, Taiwan Agricultural Research Institute, Council of Agriculture, Taichung, Taiwan, ROC.
3 Associate Research Fellow, Applied Zoology Division, Taiwan Agricultural Research Institute, Council of Agriculture, Taichung, Taiwan, ROC.

(Karny)] and *P. longiceps* Haga [= *Ophthalmothrips longiceps* (Haga)] in Taiwan. Chen (1982) recognized 10 genera and 15 species of Idolothripinae, including 6 species belonging to Idolothripini, of which *Megathrips* (= *Bactrothrips*) *honoris* Bagnall was a new record. Okajima (1979) described *Mecynothrips taiwanus* in Taiwan. Recently, he published 3 *Bactrothrips* species from Japan and Taiwan (Okajima 2006).

Species of Idolothripinae feed on fungal spores under rotten leaves on ground and on dried woody stems and branches. Their feeding behavior does not harm plants. Unlike some Terebrantian thrips, they are never agricultural pests. As with many other tiny organisms, though small in size, they play essential roles in biodiversity and contribute to environmental balance.

Diagnostic characters between two subfamilies of Phlaeothripidae (modified from Okajima, 2006)

Maxillary stylets broader, width in distal half 5–10 microns in diameter, without maxillary guides; pelta usually with 2 lateral lobes; male S2 setae on tergite IX as long as or longer than S1 setae .... Idolothripinae

Maxillary stylets narrower, width in distal half 2–3 (occasionally 3–6) microns in diameter, usually with maxillary guides or bridge; pelta usually narrow, without well-developed lateral lobes; male S2 setae on tergite IX usually short and stout .... Phlaeothripinae

Subfamily Idolothripinae Bagnall 1908

Diagnosis. Macropterous, brachypterous or apterous; female body size median to large, usually brown or dark brown. Head long or squared, usually prolonged in front of eyes; maxillary stylets broad, width in distal half 5–10 microns in diameter, V-shaped or parallel or close together in head; prosternal basantra present or absent; forewings if present, with duplicated cilia; pelta broad, usually with lateral lobes, rarely without lateral lobes, surface with sculptured reticles; tergites II–VII with 1–3 pairs of wing retaining setae; male with S2 setae on tergite IX well developed, equal to or longer than S1 setae.

MATERIALS AND METHODS

Specimens studied were mainly from 4 different sources: thrips collected by the authors in Taiwan from year 1993 to 2006, permanent slides were preserved and deposited at Taiwan Agricultural Research Institute (TARI); slides deposited at TARI by R. Takashashi; slides deposited at TARI by L. S. Chen; and slides loaned from Tokyo University of Agriculture, Japan.

Identification keys and character descriptions were made by microscope examination and comparison of these slides.

RESULTS AND DISCUSSION

Key to tribes of subfamily Idolothripinae

1 Metasternum without sternopleural sutures, pelta without campaniform sensilla, wing-retaining setae 2 pairs or more (except Hystricothripina with only one pair), tube with or without long setae .... Idolothripini

– Metasternum with or without sternopleural sutures, pelta with or without campaniform sensilla, a pair of wing-retaining setae, tube without long setae .... Pygothripini

Key to genera of tribe Idolothripini in Taiwan

1 Tube with significant lateral setae .... *Bactrothrips*

– Tube without significant lateral setae 2

2 Tergites III–V each with 3 pairs of wing-retaining setae .... *Mecynothrips*

– Tergites III–V each with 2 pairs of wing-retaining setae 3

3 Ventral side of eye longer than dorsal side .. .. *Ophthalmothrips*

– Ventral side of eye equal to or shorter than

dorsal side 4

4 Lateral lobes of pelta connected to center ... . *Elaphrothrips*

– Lateral lobes of pelta separated from center, forming 3 isolated parts; anteroangle of male mesonotum with spiracular process .... *Dinothrips*

Bactrothrips Karny (Fig. 1)

Bactrothrips Karny, 1912: 131. Type species: *Bactrothrips longiventris* Karny.

Female. Large, macropterous; head length approximately twice its width, slightly prolonged in front of eyes; setae on head blunt

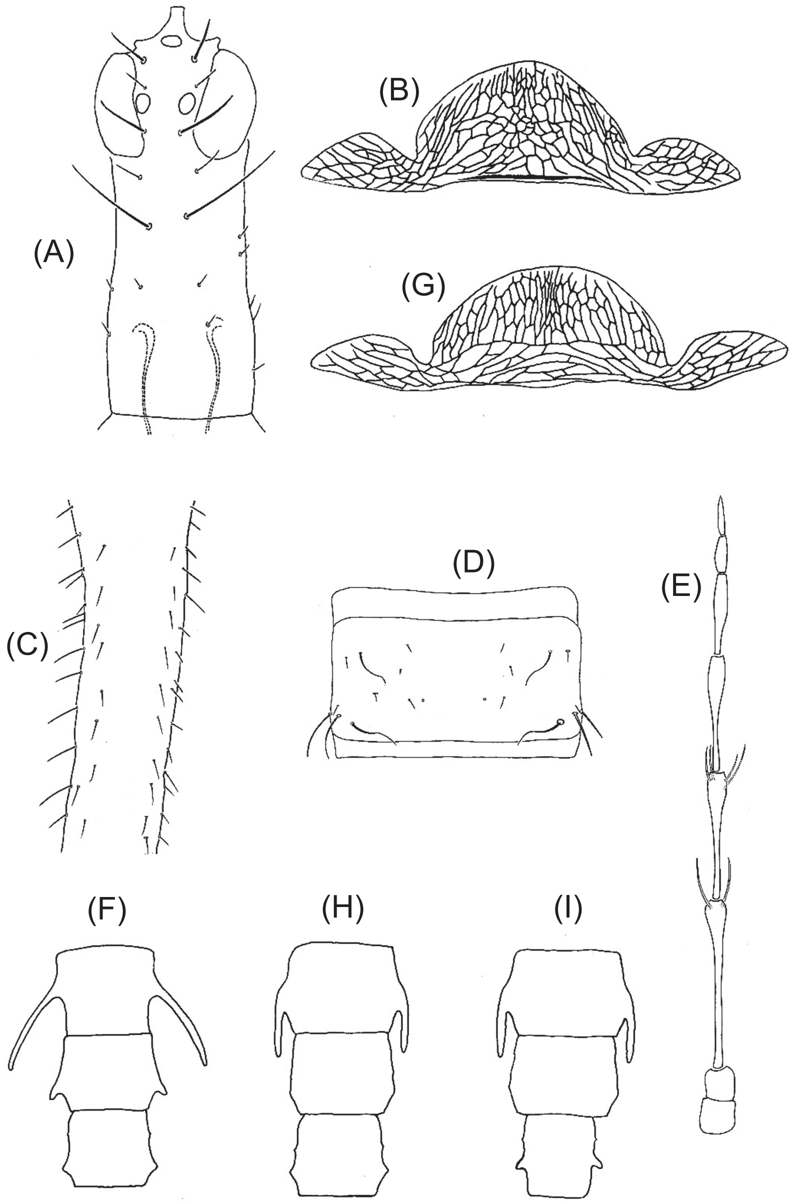

Fig. 1. *Bactrothrips* species: *brevitubus* Takahashi (Type male A–F): (A) head; (B) pelta; (C) tergite X; (D) tergite VII; (E) shape of antenna with sense cones on segments III and IV; (F) male tubercles on abdominal segments VI–VIII; (G) pelta of *honoris*; (H) tubercles of *flectoventris*; and (I) tubercles of *pictipes* and *B. honoris* (the same).

or near sharp at apex; maxillary stylets short and V-shaped; antennae slender, 8-segmented, segment III with 2, segment IV with 4 sense cones. Prothoracic basantra weak; metathoracic sternopleural sutures absent; forewing with duplicated cilia. Pelta hat-shaped, depressed between center and lateral lobes; tergites II–VII each with 2 pairs of sigmoid wing-retaining setae, without neighboring short accessory setae; tube with numerous distinct lateral setae.

Male. Macropterous, tergite VI with a pair of lateral tubercles, sometimes VII and VIII either or both with a pair of tubercles, usually smaller.

More than 40 species were recorded in the world (ThripsWiki 2018).

Key to species of *Bactrothrips* in Taiwan (based on male characters)

1 Tubercles on tergites VII finger-shaped .... *brevitubus*
– Tubercles on tergite VII triangular, not finger-shaped 2
2 Tubercles on tergite VIII triangular .... *flectoventris*
– Tubercles on tergite VIII finger-shaped 3
3 Tubercles on tergite VI brown at basal half and yellow at distal half .... *pictipes*
– Tubercles on tergite VI all brown or greyish brown at distal half .... *honoris*

Key to species of *Bactrothrips* in Taiwan (based on female characters)

1 Ventral side of eyes much longer than dorsal side; tergites II–VIII with transparent lateral spots .... *flectoventris*
– Ventral side of eyes about equal length as dorsal side; tergites without such transparent spots 2
2 Distal ends of antennal segments III–V greyish brown, obviously lighter than I and II .... *pictipes*
– Distal ends of antennal segments III–V dark brown, same color as segments I and II 3
3 Reticles on center of pelta in same direction .... *brevitubus*
– Reticles on lower 1/3 of pelta in different directions .... *honoris*

Bactrothrips brevitubus Takahashi (Figs. 1A–1F)

Bactrothrips brevitubus Takahashi, 1935:61–63.

Female. Body length 6–8 mm on slide. Color dark brown; antennal segments I and II same color as head, III and VI mostly yellow with distal ends greyish brown, segment VI basal half yellow and greyish brown distally, rest of segments greyish brown; femora brown, tibiae bicolored with brown and yellow: distal ends of fore tibiae yellow, distal 1/3 of mid tibiae yellow, hind tibiae mostly yellow with basal 1/4 brown; tube brown.

Head length 1.8–2.5 times its maximum width (Fig. 1A); antennae 8-segmented (Fig. 1E); maxillary stylets V-shaped, in posterior 1/4 of head. Forewing with 40–80 duplicated cilia, number of cilia vary depending on size of specimens. Pelta semicircular, concaved deeply between it and lateral lobes (Fig. 1B); tergites II–VII each with 2 pairs of wing-retaining setae (Fig. 1D); without abdominal tubercles; tube 1.6–2.0 times as long as head (Fig. 1C).

Male. Body length 6–8 mm on slide. Color similar to female, head length about 2.5–3 times its width; tergite VI with slender lateral tubercles, tergites VII and VIII with tubercles small and short (Fig. 1F).

Distribution. Taiwan, Japan, common in temperate and semitropical zones.

Specimens examined. Type male. 28.vii.1932; Nisinakama (Amami Oshima, Japan), S. Minowa coll.; 1F, Cingjing Farm (Nantou), dead wood, 5.vi.1993; 1F, Shaxi Forest road (Santimen, Pingtung), (coll. YS Song, 2002); 1M, Pinglin (Taipei), Litchi, 19.xi.1991; 1M, Fushan Botany Garden (Yilan), dead wood, 15.vi.1994.

Bactrothrips flectoventris Haga & Okajima (Fig. 1H)

Bactrothrips flectoventris Haga & Okajima,

1989:8–11.

Female. Body length 4–6 mm on slide. Color dark brown, antennal segments I and II brown, III–VI yellow with distal end of III to distal half of VI greyish brown, VII and VIII greyish brown; tube dark.

Head length about twice its maximum width; maxillary stylets V-shaped, in posterior 1/5 of head. Forewing with 20–43 duplicated cilia, number of cilia varies depending on body size. Center of pelta semicircular, concaved deeply to form 2 lateral lobes; without abdominal tubercles; length of tube about 1.5 times length of head.

Male. Body length 4.0–5.5 mm on slide. Color similar to female; abdominal segment VI with a pair of lateral tubercles; segment VII without tubercle; segment VIII with a pair of short tubercles (Fig. 1H).

Distribution. Taiwan, Japan.

Bactrothrips honoris (Bagnall) (Figs. 1G, 1I)

Megathrips honoris Bagnall, 1921:359.
Bactrothrips honoris (Bagnall): Mound, 1968: 136.

Female. Body length 5–7 mm on slide. Color dark brown; antennal segments I and II dark brown, III–V light yellow with distal ends dark brown, VI light yellow with distal half dark brown, rest of segments dark brown; femora brown, base and distal part of fore and mid tibiae yellow, basal and distal 1/4 of hind tibiae yellow.

Head length about 2.3 times as the maximum width; maxillary stylets V-shaped, in bottom 1/4 of head. Forewing with 40–55 duplicated cilia. Center of pelta semicircular, concaved deeply between it and lateral lobes (Fig. 1G); without abdominal tubercles; tube about 1.5 times as long as head.

Male. Body length 5–7 mm on slide. Color similar to female, head length about 2.1–2.7 times the width; tergite VI with a pair of lateral tubercles, tergite VII without lateral tubercles, tergite VIII with a pair of small thumb-like tubercles (Fig. 1I)

Distribution. Taiwan, Japan.

Specimens examined. 2F, Shenkeng (Taipei), *Ehretia longiflora*, 10.v.1978; 2F, Nan Shih Creek (Taipei), grasses, 23.iii.1995; 1F, Biyun temple (Tainan), dead leaves, 5.v.1993; 1F, Maopu (Taipei), Bamboo, 16.xii.1993; 1M, Lanhuschi (Nantou), Fern, vii.1993.

Bactrothrips pictipes Haga & Okajima (Fig. 1I)

Bactrothrips pictipes Haga & Okajima, 1989: 15–18.

Female. Body length 4.8–6.3 mm on slide. Color dark brown; distal ends of hind femora yellow, tibiae bicolored with yellow bases; antennal segments I and II brown, bases of III–VI yellow but brown distally, obviously lighter than I or II.

Head length about twice its maximum width; interocellar setae longest on dorsum; maxillary stylets V-shaped, in posterior 1/3 of head; forewing with 29–47 duplicated cilia. Center of pelta semicircular, concaved deeply between it and lateral lobes; without abdominal tubercle; tube about 1.5 times as long as head.

Male. Body length 4–6 mm on slide. Color similar to male; tergite VI with a pair of lateral tubercles, tergite VII without lateral tubercles, tergite VIII with a pair of small thumblike tubercles (Fig. 1I).

Distribution. Taiwan, Japan.

Specimens examined. 1F, Wufeng (Taichung), Mongo, 21.i.1991; 1F, 99 Peaks (Nantou), Litchi, 19.xi.1991.

Dinothrips Bagnall (new record)

Dinothrips Bagnall, 1908b:190. Type species: *Dinothrips sumatrensis* Bagnall.
Paxillothrips Ananthakrishnan, 1961:250; Palmer & Mound, 1978:166.

Female. Large, macropterous, dark in color; head length about twice its width, prolonged in front of eyes, cheeks with strong setae; antennae 8-segmented, segments III and IV with 2 and 4 sense cones, respectively;

maxillary stylets V-shaped. Prothoracic basantra weak; metathoracic sternopleural sutures absent; forewing with duplicated cilia; femora with numerous setae, tarsal tooth present on both male and female. Lateral lobes of pelta separated from center to form 3 isolated parts; 2 pairs of wing-retaining setae on each of tergites II–VII, several pairs of smaller accessory setae; tube straight-sided, without conspicuous lateral setae.

Male. Fore femora enlarged, with numerous setae; foretarsal tooth larger than female; mesothorax with spiracular processes, reduced or absent in some species.

Five species were recorded in the world.

Dinothrips spinosus (Schmutz) (Fig. 2)

Ischyrothrips spinosus Schmutz, 1913:1078.

Female. Body length 6–8 mm on slide. Color dark brown; antennae dark brown, except segment III mainly yellow with both ends brown; forewings hyaline, veins brown; tube brown with posterior end yellow.

Head length about 1.8–2.0 times of its

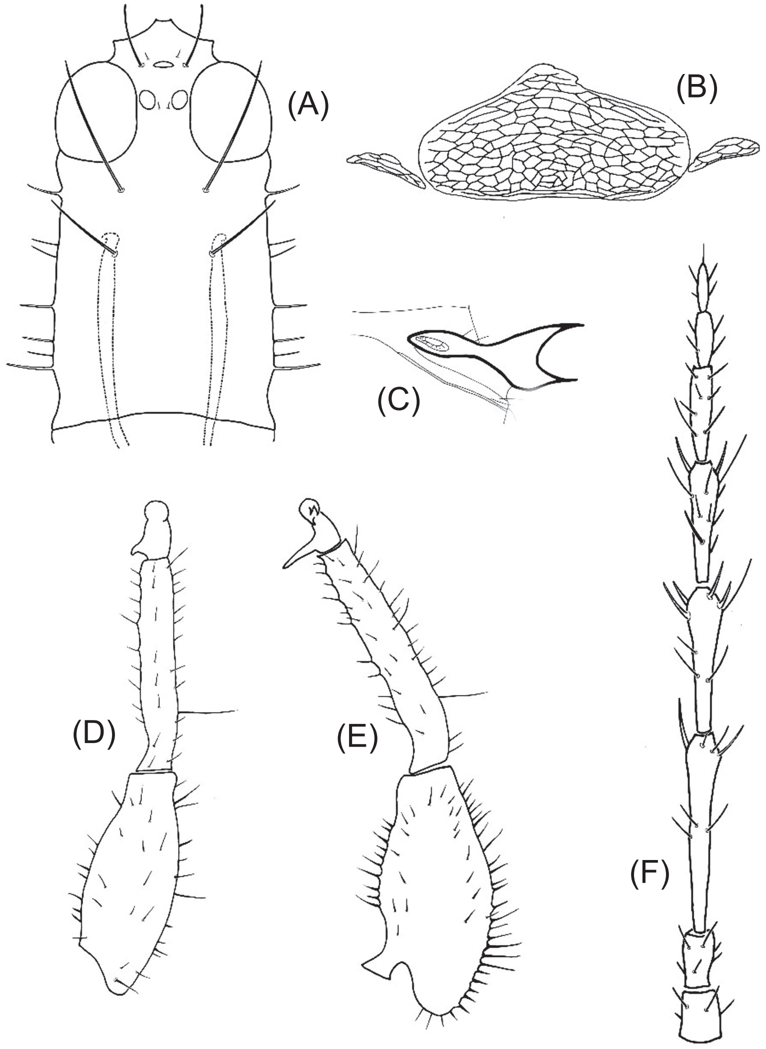

Fig. 2. *Dinothrips spinosus* (Schmutz) (female except C & E): (A) head; (B) pelta; (C) anterolateral folk process on male mesonotum; (D) fore leg of female; (E) fore leg of male; and (F) antenna.

width (Fig. 2A), cheeks with 7–8 strong setae; interocellar setae lateral to fore ocellus; postocular setae I longest on dorsum, postocular setae II second longest; antennae 8-segmented (Fig. 2F); maxillary stylets retracted to middle of head; one vertical seta on middle of fore femur, and one on basal 1/3 of tibia; foretarsal tooth hook-shaped (Fig. 2D). Pelta anterior margin irregular, lateral lobes separated completely from center (Fig. 2B); tube as long as head.

Male. Anteroangle of mesothorax with spiracular process (Fig. 2C), it may be small or lacking on some specimens; fore femora enlarged, with numerous strong setae on granular bases; fore tarsi with slender tooth (Fig. 2E).

Distribution. Taiwan, Mainland China, Myanmar, India, Sri Lanka, Indonesia.

Specimens examined. 2F3M, Wufeng (Taichung), dead *Phoenix*, 23.vii.1993.

Elaphrothrips Buffa (Fig. 3)

Elaphrothrips Buffa, 1909:162–163. Type spe-

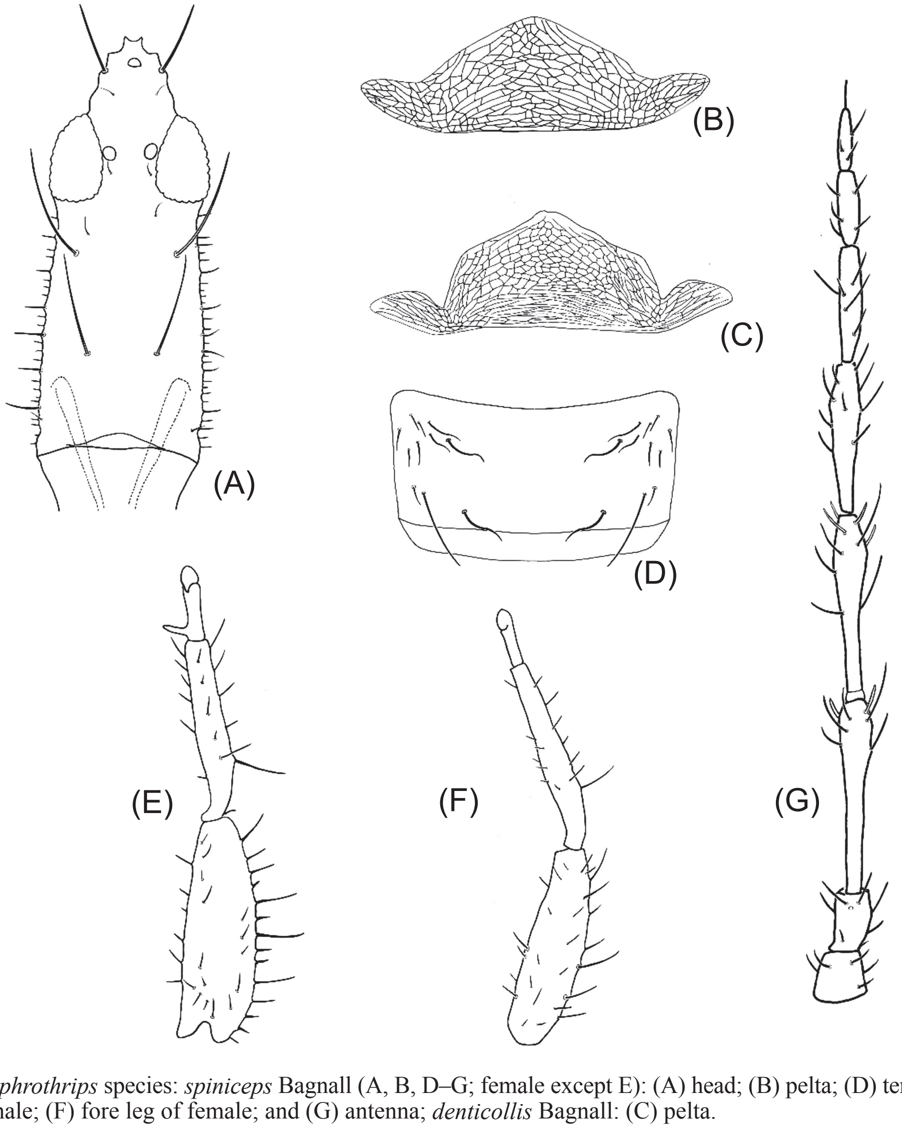

Fig. 3. *Elaphrothrips* species: *spiniceps* Bagnall (A, B, D–G; female except E): (A) head; (B) pelta; (D) tergite III; (E) fore leg of male; (F) fore leg of female; and (G) antenna; *denticollis* Bagnall: (C) pelta.

cies: *Idolothrips coniferarum* Pergande.

Female. Large, macropterous, head long, distinctly prolonged in front of eyes; ventral side of eyes rarely longer than dorsal side; posterior ocelli situated on inner sides of eyes, close or connected to eyes; interocellar setae long, situated posterolateral to anterior ocellus, postocular setae long; antennae 8-segmented, segments III and IV with 2 and 4 sense cones, respectively; maxillary stylets V-shaped. Prosternal basantra present, metathoracic sternopleural sutures absent; forewing with numerous duplicated cilia; foretarsal tooth present or absent. Pelta with 2 lateral lobes; tergites II–VI each with 2 pairs of wing-retaining setae and several pairs of short accessory setae; tube straight-sided, without conspicuous lateral setae.

Male. Fore femur larger than that of female, often with a sickle-shaped seta on outer of apical margin; fore tibia without distal spur, foretarsal tooth present.

About 120 species in the world.

Key to species of *Elaphrothrips* in Taiwan

1 Antennae brown, except segment III yellow; female foretarsal tooth small; male fore femur enlarged .... *denticollis*

2 Antennae brown, segment III yellow, basal 1/3–1/2 of both segments IV and V yellow; female foretarsal tooth lacking; male fore femur normal .... *spiniceps*

Elaphrothrips denticollis Bagnall (Fig. 3C)

Elaphrothrips denticollis Bagnall, 1909:527.
Elaphrothrips mucronatus Priesner, 1935a:167; Mound & Palmer, 1983:66
Elaphrothrips sumbanus Priesner, 1935a:169; Mound & Palmer, 1983:66
Elaphrothrips productus Priesner, 1935a:170; Mound & Palmer, 1983:66

Female. Body length 6 mm on slide. Color dark brown; antennae dark brown, except segment III yellow with distal end brown; major setae grayish.

Head length about 1.8 times its width, eyes bulged, cheeks with about 8 strong setae; postocular setae I longest on dorsum, postocular setae II long; maxillary stylets in posterior 1/5 of head. Prosternal basantra large; forewing with 45 duplicated cilia; fore femur with numerous setae, tarsal tooth small and triangular. Pelta concaved between center and lateral lobes (Fig. 3C); tergites II–VI each with 2 pairs of wing-retaining setae, and surrounded by 2–5 pairs of accessory setae; tube length about 0.85 times of head.

Male. Dark brown, similar to female; antennal segments III–V with 2–3 brown, stout and straight setae; fore femur enlarged, with numerous stout setae, often with a sickle-shaped seta on outer apical margin; fore tarsus with slender tooth.

Distribution. Taiwan, Mainland China, India, Burma, Indonesia, Malaysia.

Specimens examined. 3F, Fenggu (Wufeng, Taichung), dead leaves of *Dimocarpus longan*, 23.vii.1993; 1M, same data as above but on dead leaves of *Vercinia* sp., 23.vii.1993; 1F1M, Lianhuachi (Nantou), *Vernicia* sp., 9.vii.1993; 1F1M, Loanong (Kaohsiung), grasses, 22.vii.1992; 1F, Kuantzuling (Tainan), *Cerasus* sp., 1.iv.1993; 1M, TARI (Taichung), *Hibiscus mutabilis*, 22.viii.1990.

Elaphrothrips spiniceps Bagnall (Figs. 3A, 3B, 3D–3G)

Elaphrothrips spiniceps Bagnall, 1932:514–515.
Elaphrothrips graveleyi Bagnall, 1934:628–630; Mound & Palmer, 1983:68
Elaphrothrips clarispinis Priesner, 1935a:247–249; Mound & Palmer, 1983:68

Female. Body length 6 mm on slide. Color dark brown; antennae brown, segment III yellow except distal end grayish brown; basal 1/3–1/2 of segment IV and V yellow; legs dark brown, except fore tibiae yellowish brown; mid and hind tibiae distal end yellowish; major setae light.

Head length about 2.7 times of its width

(Fig. 3A), eyes bulged, cheeks with about 8 strong setae; postocular setae I longest on dorsum, postocular setae II long; antennae 8-segmented (Fig. 3G); maxillary stylets in posterior part of head. Prosternal basantra large; forewing with 35–40 duplicated cilia; fore femur with numerous setae, tarsus without tooth (Fig. 3F). Pelta triangular, lateral lobes well connected with center (Fig. 3B); tergites II–VI each with 2 pairs of wing-retaining setae, and surrounded by 2–5 pairs of accessory setae (Fig. 3D); tube length about 0.85 times of head.

Male. Dark brown, similar to female; antennal segments III–V with 2–3 brown stout setae; fore femur not enlarged, with numerous stout setae, a sickle-shaped seta on outer apical margin; fore tarsus with slender tooth (Fig. 3E).

Specimens examined. 1F, *E. falcatus* Karny, Formosa: Shinten, on *Psidium*, 13.vi.1931; 1M, *E. falcatus*, on *Artocarpus*, i.1926; 2F, *E. falcatus*, on *Artocarpus integrifolia*, i.1928. 3F8M, Guanziling (Tainan), dried leaves, 4.v.1999; 5F2M, Wuxi Bridge (Nantou), dried leaves of Mongo, 25.v.1999; 3M, Guanziling (Tainan), Peach, 4.v.1999; 1F3M, Guanziling (Tainan), *Ficus Harlandii* Benth., 4.v.1999; 1F, Wutai (Pingtung), Willow, 9.v.2002; 1M, Wutai (Pingtung), *Areca catechu*, 9.v.2002; 1F, Kending (Pingtung), *Phragmites communis*, 20.xii.2000; 1M, Fenggu (Wufeng, Taichung), Tung oil tree (dead wood), 23.vii.1993; 1M, TARI (Taichung), *Hibiscus mutabilis*, 22.viii.1990; 1M, Laonung (Pingtung), grasses, 22.iv.1992; 1M, Lianhuachi (Nantou), *Macaranga tanarius*, 9.vii.1993.

Distribution. Taiwan, India, Nepel, Burma, Indonesia.

Remark. All specimens in TARI (6 females and 2 males) labled as *Elaphrothrips falcatus* Karny by Takahashi were identified as *E. spiniceps* by Chen (1982).

Mecynothrips Bagnall (Fig. 4)

Mecynothrips Bagnall, 1908a: 356. Type species: *Mecynothrips wallacei* Bagnall.

Phoxothrips Karny; Haga & Okajima, 1974:375–384.

Female. Large, macropterous; head length more than twice its width, prolonged in front of eyes; ventral surface of eyes no longer than dorsal surface; posterior ocelli very close to eyes; maxillary stylets short, V-shaped, low in head; antennae 8-segmented, segments III and IV with 2 and 4 sense cones, respectively. Prosternal basantra present; metathoracic sternopleural sutures absent; fore tarsus without tooth, forewing with multiple duplicated cilia. Pelta with 2 lateral lobes; abdominal tergite II with 2 pairs of wing-retaining setae, and several pairs of accessory setae; tergites III–V each with 3 pairs of wing-retaining setae and several pairs of accessory setae; tube straight-sided, without conspicuous lateral setae.

Male. Fore femur enlarged, inner side with 1 or 2 teeth; fore tibia often with a distal spur, foretarsal tooth present. Fourteen species were recorded in the world.

Mecynothrips pugilator (Karny) (Figs. 4B–4H)

Phoxothrips pugilator Karny, 1913a: 132–134; Haga & Okajima, 1974:379–382.

Elaphrothrips takahashii Priesner, 1935b:372–373; Mound & Palmer, 1983:70.

Mecynothrips pugilator (Karny); Palmer & Mound, 1978, 207.

Female. Body length 6.4–8.5 mm on slide. Color dark brown; antennal segments dark brown, except bases of III–V and distal end of VI yellow, VII and VIII in lighter brown than I and II; forewings hyaline with apical ends greyish brown; femora and tibiae brown; except distal ends greyish, tarsi yellow.

Head length about 2.5 times its maximum width (Fig. 4B), distinctly prolonged in front of eyes, anterior ocellus situated on this protrusion; antennae 8-segmented (Fig. 4H); maxillary stylets V-shaped, reaching posterior 1/6 of head. Prosternal basantra present (Fig. 4D), notol setae blunt; metathoracic stenopleural sutures absent; forewing with near 50 dupli-

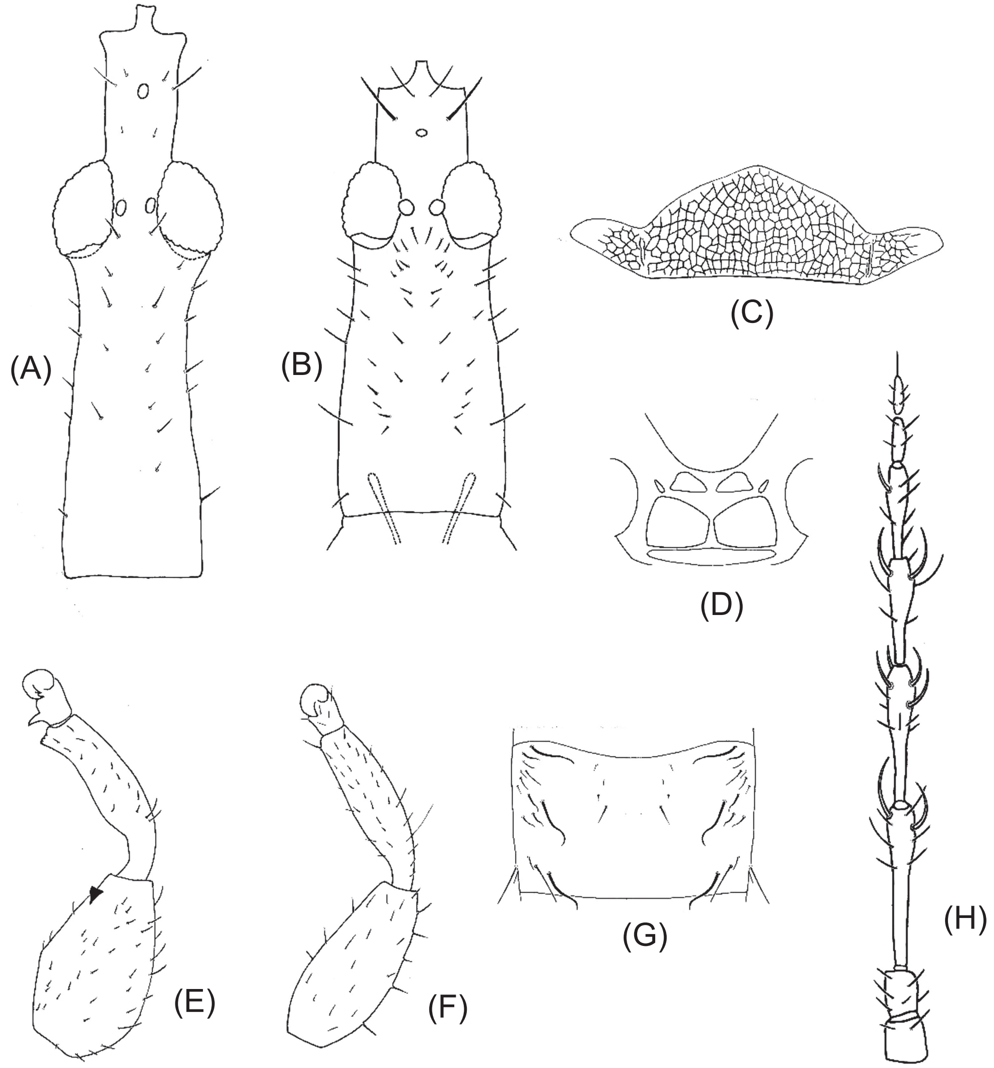

Fig. 4. *Mecynothrips* species: *taiwanus* Okajima: (A) head; *pugilator* Haga & Okajima: (B) head; (C) pelta; (D) prothoracic prepectal plates; (E) male fore leg; (F) female fore leg; (G) tergite III; and (H) antenna.

cated cilia; fore femur always narrower than head; foretarsal tooth absent (Fig. 4F). Lateral lobes of pelta well connected with center (Fig. 4C); tergite II with 2 pairs of wing-retianing setae, and several pairs of short accessory setae; tergites III–V each with 3 pairs of wing-retaining setae and several pairs of short accessory setae; tube straight-sided, without conspicuous lateral setae, about 0.8 times the length of head.

Male. Body length 5.7–11.5 mm, color similar to female, femur enlarged especially on large males, with a dark spur on inner distal portion, tibia with a distal spur, tarsal tooth present (Fig. 4E).

Distribution. Taiwan, Japan.

Specimens examined. 1M, *M. pugilator* (K.), Tapu (Chiayi Hsien), on Cassava (dead twig), 10.ii.1980, coll. Y. H. Tseng; 1F4M, Wanfeng (Taichung), dead branches, iii.2006; 1F, Cingjing Farm (Nantou), Banana, 5.vi.1996; 1M, Kending (Pingtung), *Cunninghamia lanceolate*, 13.i.1993.

Mecynothrips taiwanus Okajima (Fig. 4A)

Mecynothrips taiwanus Okajima, 1979:127–130.

Male. Body length 13–17 mm. Color dark brown; antennal segments dark brown, except bases of III–V pale yellow; forewings hyaline with apical ends greyish brown; femora and tibiae brown at base, yellow at distal ends.

Head length about 3.3–4.0 times its maximum width (Fig. 4A), clearly prolonged in front of eyes, anterior ocellus situated on this protrusion; maxillary stylets V-shaped, in posterior part of head. Prosternal basantra present, pronotal setae blunt; metathoracic stenopleural sutures absent; fore femur always narrower than head, foretarsal tooth absent. Lateral lobes of pelta well connected with center; tergite II with 2 pairs of wing-retaining setae, and several pairs of short accessory setae; tergites III–V each with 3 pairs of wing-retaining setae and several pairs of short accessory setae (Fig. 4G); tube straight-sided, without conspicuous lateral setae; shorter than head (with preocular projection), length about 0.8 times its of head.

Female. Unknown.

Distribution. Taiwan.

Remarks. This species is similar to *M. pugilator* in having a hump medially on inner margin of fore femur and seta bearing subapical projection of fore tibia. However, *taiwanus* can be distinguished from the latter by: preocular projection longer; cheeks distinctly constricted behind eyes; eyes larger; pronotal setae shorter; foretarsal tooth curved posteriorly; pronotum slightly projected anterolaterally (Okajima 1979).

Specimen examined: 1 male paratype, lienhuachi (Nantou), dead leaves, 6.v.1977 (Okajima).

Ophthalmothrips Hood

Ophthalmothrips Hood, 1919:67. Type species: *Ophthalmothrips pomeroyi* Hood.

Pyrgothrips Karny, 1924:35; Mound, 1974:89.

Female. Medium to large, macropterous or brachypterous. Head long, prolonged in front of eyes, ventral side of eyes obviously longer than dorsal side; antennae 8-segmented, segments III and IV with 2 and 4 sense cones, respectively; maxillary stylets V-shaped. Prosternal basantra present, metathoracic sternopleural sutures absent; fore femora normal, foretarsal tooth absent; forewing with duplicated cilia. Pelta triangular; tergites II–VI each with 2 pairs of wing-retaining setae, and several pairs of small accessory setae; tube straight-sided, no conspicuous lateral setae.

Male. Shape similar to female, fore femur not enlarged, foretarsal tooth absent.

Ten species were recorded in the world.

Ophthalmothrips formosanus (Karny)

Idolothrips formosanus Karny, 1913b:130–131.

Pyrgothrips formosanus (Karny); Haga, 1975: 270.

Elaphrothrips formosanus (Karny): Priesner, 1935a:64–66

Ophthalmothrips formosanus (Karny); Mound & Palmer, 1983:71.

Female. Body length 2.4 mm on slide. Black colored; head length about 2.3 times of its width, preocular part short, square; ventral extension of eyes developed, posterolateral corner of eyes not produced; postocular setae long; cheeks somewhat swollen; foretarsal tooth lacking, forewing with 12 duplicated cilia; tube length about 0.8 times of head.

Male. Unknown.

Distribution. Taiwan.

Ophthalmothrips longiceps (Haga) (Fig. 5)

Pyrgothrips longiceps Haga, 1975:270–273.

Ophthalmothrips longiceps (Haga); Mound & Palmer, 1983:71.

Female. Body length 4.0–4.5 mm on slide. Color dark brown; antennae segments I and II dark brown; III–V yellow, except both ends of III, distal 1/2 of IV, and distal 1/3 of V brown; VI–VIII dark brown; forewings hyaline; margins of fore tibiae yellow.

Head length about 2.5 times its maximum width (Fig. 5A), prolonged in front of

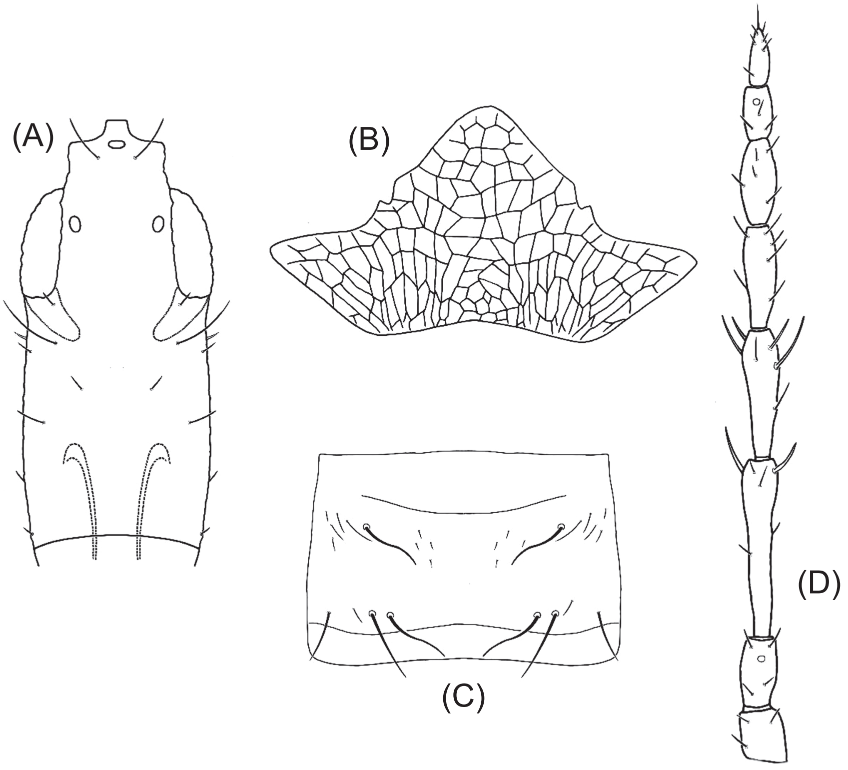

Fig. 5. *Ophthalmothrips longiceps* (Haga) (female): (A) head; (B) pelta; (C) tergite III; and (D) antenna.

eyes; postocelli located between anterior half of eyes; antennae 8-segmented (Fig. 5D); maxillary stylets extend to about 1/3 of head. Pronotal setae sharp or blunt, foretarsal tooth absent; forewing with 13–15 duplicated cilia. Two lateral lobes of pelta well connected with main lobe (Fig. 5B); tergites II–VI each with 2 pairs of wing-retaining setae, and several pairs of small accessory setae (Fig. 5C); tube straight-sided, about 1.5 times as long as head, no conspicuous lateral setae. Ten species were recorded in the world.

Male. Body length 3–4 mm on slide. Color similar to females; interocellar setae on posterolateral of anterior ocellus; foretarsal tooth absent; forewing with 12 duplicated cilia.

Distribution. Taiwan, Japan.

Specimens examined. 1M, *P. longiceps* Haga, Pali (New Taipei City), *Psychotria rubra*, 7.x.1978; 1M, Houli (Taichung), Grass, 25.xi.1978; 1F2M, NanJan Mt. (Kending, Pingtung), wood, 22.xi.2001; 4F6M, Chishan (Kaohsiung), grasses, 21.x.1992; 1M, Kungziling (Tainan), dead wood, iii.1993.

ACKNOWLEDGEMENTS

The authors thank Dr. M. Masumoto, Plant Protection Station of Japan, for the loan of the thrips specimens deposited at Tokyo University of Agriculture. We thank Dr. C. F. Lee, insect taxonomist of TARI, for his suggestions and sharing the drawing techniques. Thanks are due to Dr. L. A. Mound, CSIRO of Australia, for giving valuable comments.

REFERENCES

Ananthakrishnan, T. N. 1961. New tubuliferan Thysanoptera from India. J. Zool. Soc. India 12:250–258.

Bagnall, R. S. 1908a. On some new and curious Thysanoptera (Tubulifera) from Papua. Ann. Mag. Nat. Hist. 1:355–363.

Bagnall, R. S. 1908b. On some new genera and species of Thysanoptera. Trans. Nat. Hist. Soc. Northumberland 3:183–217.

Bagnall, R. S. 1909. On some new and little-known exotic Thysanoptera. Trans. Nat. His. Soc. Northumberland 3:524–540.

Bagnall, R. S. 1921. XLI.- Brief descriptions of new Thysanoptera- XI. Ann. Mag. Nat. His. 7:355–368.

Bagnall, R. S. 1932. LXI.- Brief descriptions of new Thysanoptera- XVII. Ann. Mag. Nat. His. 10:505–520.

Bagnall, R. S. 1934. LXIV.- Contributions towards a knowledge of the Thysanopterous genus *Elaphrothrips* Buffa.- I. Ann. Mag. Nat. His. 13:627–636.

Buffa, P. 1909. I Exotic thrips in the Civial Museum of Natural History of Genoa. (I Tisanotteri esotici nel Museo Civico di natural di Genova). Redia 5:157–172. (in Italian)

Chen, L. S. 1982. Studies on the subfamily Idolothripinae (Thysanoptera: Phlaeothripidae) in Taiwan. NTU Phytopathol. Entomol. 9:53–67.

Dang, L. H. and G. X. Qiao. 2013. Review of the spore-feeding Idolothripinae from China. Zookeys 345:1–28.

Eow, L. X., L. A. Mound, and Y. F. Ng. 2011. Genera of spore-feeding Thysanoptera from Southeast Asia (Phlaeothripidae, Idolothripinae), with a species checklist from Peninsular Malaysia. Zootaxa 2928:1–19.

Haga, K. 1975. A revision of the genus *Pyrgothrips* Karny with keys to the world species (Thysanoptera, Tubulifera). Kontyû 43:263–280.

Haga, K. and S. Okajima. 1974. Redescription and status of the genus *Phoxothrips* Karny (Thysanoptera). Kontyû 42:375–384.

Haga, K. and S. Okajima. 1989. A taxonomic study of the genus *Bactrothrips* Karny (Thysanoptera, Phlaeothripidae) from Japan. Bull. Sugadaira Montane Res. Cen. 10:1–23.

Hood, J. D. 1919. On some new Idolothripidae (Thysanoptera). Insec. Inscit. Menstr. 7:66–74.

Karny, H. 1912. Some more Tubulifera from tropical Africa (Einige weitere Tubuliferen aus dem tropischen Africa). Entomologische Rundschau 29:130–133, 138–139, 150–151. (in German)

Karny, H. 1913a. Contribution to the Thysanoptera fauna of New Britain. (Beitrag zur Thysanopteren-Fauna von Neu-Britannien). Archiv für Naturgeschichte 79(1):123–136. (in German)

Karny, H. 1913b. H. Sauter's Formosa-Collection (H. Sauter's Formosa-Ausbeute). Supplementa Entomologica 2:127–134. (in German)

Karny, H. 1924. Results of Dr. E. Mjoberg's Swedish scientific expeditions to Australia 1910–1913. 38. Thysanoptera. Arkiv fur Zoologi 17A(2):1–56.

Majid, M. B., X. I. Tong, J. N. Feng, and X. X. Chen. 2011. Thrips of China. J. Spec. Dist. 7:720–744.

Mound, L. A. 1968. A review of R. S. Bagnall's Thysanoptera collections. Bull. Br. Mus. Suppl. Nat. His. 11:1–181.

Mound, L. A. 1974. Spore-feeding thrips (Phlaeothripidae) from leaf litter and dead wood in Australia. Australian J. Zool. Suppl. 27:1–106.

Mound, L. A. and J. M. Palmer. 1983. The generic and tribal classification of spore-feeding Thysanoptera (Phlaeothripidae: Idolothripinae). Bull. Br. Mus. Nat. His. 46:1–174.

Okajima, S. 1979. Notes on the Thysanoptera from Southeast Asia, VI. A new species of the genus *Mecynothrips* from Taiwan (Phlaeothripidae). Trans. Shikoku Entomol. Soc. 14:127–130.

Okajima, S. 2006. The Insects of Japan, Vol. 2: The Suborder Tubulifera (Thysanoptera). Touka Shobo Co. Ltd. Fukuoka, Japan. 720 pp.

Palmer J. M. and L. A. Mound. 1978. Nine gerera of fungus-feeding Phlaeothripidae (Thysanoptera) from the oriental region. Bull. Br. Mus. Nat. Hist. (Ent.) 37:153–215.

Priesner, H. 1935a. Indomalayan Thysanoptera (Indomalayische Thysanopteren) VI. Konowia 14:58–67, 159–174, 241–253, 323–339. (in German)

Priesner, H. 1935b. New or little-known oriental Thysanoptera. Philippine J. Sci. 57:351–375.

Schmutz, K. 1913. To know the Thysanoptera fauna of Ceylon. (Zur kenntnis der Thysanoperenfauna von Ceylon). Sitzungsberichte der Akademie der Wissenschaften mathematisch-naturwissenschaftliche Klasse 122:991–1089. (in German)

Takahashi, R. 1935. An interesting thrips from Amami-Oshima, Loochoos. Mushi 8:61–63.

ThripsWiki. (2018). ThripsWiki - providing information on the world's thrips. http://thrips.info/wiki/Main_Page (visit on 1/9/2018)

台灣寬薊馬亞科 (纓翅目：管尾亞目：管尾薊馬科) 之分類描述 I 雙毛族

王清玲 [1]　陳怡如 [2]　林鳳琪 [3,*]

摘要

王清玲、陳怡如、林鳳琪。2018。台灣寬薊馬亞科 (纓翅目：管尾亞目：管尾薊馬科) 之分類描述 I 雙毛族。台灣農業研究 67(4):323–336。

本文記錄與描述台灣寬薊馬亞科 (Idolothripinae) 雙毛族 (Idolothripini) 5 屬 11 種之分類特徵，族內每屬與同屬內之每種均製作檢索表，其中胸叉薊馬 *Dinothrips spinosus* (Schmutz) 為台灣新紀錄屬與種。

關鍵詞：分類、纓翅目、管薊馬科、寬薊馬亞科、台灣。

投稿日期：2018 年 3 月 4 日；接受日期：2018 年 5 月 13 日。
[*] 通訊作者：fclin@tari.gov.tw
[1] 農委會農業試驗所應用動物組前研究員。台灣 台中市。
[2] 農委會農業試驗所應用動物組助理研究員。台灣 台中市。
[3] 農委會農業試驗所應用動物組副研究員。台灣 台中市。

台灣農業研究 (J. Taiwan Agric. Res.) 67(4):337–346 (2018)
DOI:10.6156/JTAR.201812_67(4).0002

Research Article

Reduction in Genetic Variability for Quantitative Trait Prescutellar Bristle Number of Melon Fly [*Bactrocera* (*Zeugodacus*) *cucurbitae* (Coquillett)]

Edward Yun Cheng[1], Zu-Hsien Wang[2], Yu-Bing Huang[3,*], Ming-Yaw Chiang[3], Hsiu-Ying Lu[4], Dong-Hong Wu[5], Chung-Min Yang[2], and Chun-Chi Nien[2]

Abstract

Cheng, E. Y., Z. H. Wang, Y. B. Huang, M. Y. Chiang, H. Y. Lu, D. H. Wu, C. M. Yang, and C. C. Nien. 2018. Reduction in genetic variability for quantitative trait prescutellar bristle number of melon fly [*Bactrocera* (*Zeugodacus*) *cucurbitae* (Coquillett)]. J. Taiwan Agric. Res. 67(4):337–346.

The number of prescutellar bristles (prsc) in melon fly, *Bactrocera* (*Zeugocacus*) *cucurbitae* (Coquillett), is quantitative genetic controlled. The polygenic characters are usually expressed in the continuous traits, but the prsc polymorphism in melon flies are limited to only two phenotypes, 2 prsc phenotype (2PHT) and 4 prsc phenotype (4PHT) at 95% and 5% of the field population, respectively. How a single phenotype dominated a complete prsc trait in genetics and this phenotype happen to have the fewest prsc number among all phenotypes, and how the prsc variability is reduced may be the first question to be studied. A multiple prsc (MB) strain with 6 to 19 prsc bristles and operated only on additive quantitative genetics has been chosen to compare the prsc variability of 2PHT. Both 2PHT and 12PHT were separately single paired with 6, 8, 10, and 12 prsc phenotypes in two serial crosses and the prsc phenotypes of their progeny were analyzed for possible clues. In 2PHT serial crosses, the prsc mean (PM) varied from 3.6 to 4.7 in a 4–8 prsc midparent (MP) window, the biological buffering effect replaced the additive variation of quantitative genetics, as the variation of PM is buffered within 1.1 prsc. In MB series, within a MP window of 9–12 prsc, the PM varied additively from 8.5 to 12.0 prsc without prsc number reduction. The buffering and additive effect of prsc with respected to 2PHT and 12PHT serial crosses were illustrated in the PM–MP map (modified from the genotype-phenotype map or G-P map). The comparative study confirmed that 2PHT is the genetic source of prsc variability reduction. By the backcross tests of 2PHT progeny, the heritability and the cumulative effect of prsc reduction were observed. A simulation of random mating between 2PHT and MB strain was conducted, 2PHT and MB strains mated freely and the reproductive advantage was estimated at 3/2 for 2PHT, another possible factor in reducing the prsc number and variability. Once MB strain mated with 2PHT, the prsc variability reduction could be functional in stabilizing selection for generations, as the observation of four generations of sibling mating, the PM lowered from 4.9 to 3.2, the variance lowered from 15.0 to 2.7, and prsc phenotype trait shortened from 19 to 11 bristles.

Key words: Quantitative genetic, Prescutellar bristle, Melon fly.

Received: July 19, 2017; Accepted: May 30, 2018.

* Corresponding author, e-mail: ybhuang@tari.gov.tw
1 Former Research Fellow, Applied Zoology Division, Taiwan Agricultural Research Institute, Taichung, Taiwan, ROC.
2 Technicians, Applied Zoology Division, Taiwan Agricultural Research Institute, Taichung, Taiwan, ROC.
3 Assistant Research Fellow, Applied Zoology Division, Taiwan Agricultural Research Institute, Taichung, Taiwan, ROC.
4 Director, Miaoli District Agricultural Research and Extension Station, Miaoli, Taiwan, ROC.
5 Assistant Research Fellow, Crop Science Division, Taiwan Agricultural Research Institute, Taichung, Taiwan, ROC.

INTRODUCTION

In Drosophila, the scute mutant, a fly that lacked a few large bristles (macrochaetes) has been started serial studies of different alleles removed different subsets of bristles, and the investigation led to the important finding of achaete-scute gene complex (AS-C). The molecular dissection of AS-C provides support to the claim that scute can be subdivided into several smaller regions, each is responsible for the development of one or a few macrochaetes (Ghysen & Dambly-Chaudière 1988). Another important feature of bristle development is the generation of species-specific two-dimensional patterns. On each half of the dorsal mesothorax of *Drosophila melanogaster*, 11 macrochaetes occupy precisely constant positions (Lindsley & Zimm 1992). The evolution of bristle number and patterns in Diptera were extensively compared and proposed that all species display four rows macrochaetes on mesothorax as they are acrostichal (AC), dorsocentral (DC), intra-alar (IA), and supra-alar (SA) bristles (Simpson *et al*. 1999; Simpson & Marcellini 2006). In the Acalyptrata, there is usually only a single postsutural AC bristle, called the prescutellar (Simpson *et al*. 1999). The prescutellar belonged to the achaete sets of bristles in Drosophila (Garcia-Bellido 1979), and regulated by T5 gene on the AS-C chromosome segment in the molecular and phenotypic map (Ghysen & Dambly-Chaudière 1988). The melon fly, *Bactrocera* (*Zeugodacus*) *cucurbitae* (Coquillett), is recorded with 2 prescutellar bristles (prsc) (White & Elson-Harris 1992), and some 4 prsc specimens are collected in the fruit fly control project, since fruit fly is the international quarantine pest, any new, unidentified or invaded species will impose severe threat on fruit production and trade. It becomes necessary to clarify whether there is another pest species or just the prsc polymorphic variant of melon fly. The laboratory study confirmed that 2 prsc phenotype (2PHT) and 4 prsc phenotype (4PHT) interbreed normally. The study of prsc polymorphism started in 1994 with the question of why a small portion of individuals with 4PHT exists in a wild population with most of individuals exhibiting 2PHT. After concluding the prsc is quantitative genetic controlled (Cheng *et al*. 2014), the same question takes a turn; as why a single 2PHT phenotype dominates a continuous prsc trait, which contradicts to the principle of quantitative genetics (Emerson & East 1913). The domination of the 2PHT does not come without a reason. However, very little is known about the function of notal bristles; and therefore, it is difficult to speculate on the nature of such selective pressures (Simpson *et al*. 1999) and the variability of polygenic prsc of MB strain must be genetically reduced to only two phenotypes. The 2PHT surge (Cheng *et al*. 2018) in the wild strain can contribute 22% increase in the second filial (F_2) generation and it is not enough to explain the 2PHT domination. The most discussed wild type robustness concept in quantitative genetics is the epigenetic canalization theory proposed by Waddington (1942, 1947). The canalization concept in population genetics has been illustrated more detail by Ridley (2011) as "canalized relation" and Hansen (2006) related the genotype-phenotype map to canalization. The non-linear relationship between genotype and phenotype produced remarkable response to selection. Particularly on the variability reduction, Gibson & Wagner (2000) defined the biological systems evolve to a state of higher stability against mutational and environmental perturbations, through the reduction in variability of a trait. In this study, the prsc variability was examined by crossing the wild type to other selected strains for the evidence of 2PHT robustness.

MATERIALS AND METHODS

Insect materials

The insect rearing, selection and the definition of bristles (White & Elson-Harris 1992; Drew & Hancook 1994) were the same as described in the previous report (Cheng *et al*.

2014).

(1) The 2 prsc (2P) phenotype strain was selected from the laboratory reared wild strain (Cheng *et al*. 2014), the prsc mean = 2.0 ± 0.17, and the prsc traits = 1–4.

(2) The multiple prscs (MB) strain was selected from a laboratory reared 4PHT strain (Cheng *et al*. 2014), the prsc mean = 12.3 ± 1.9, and the prsc traits = 6–19.

The prsc variability test

The prsc genetic variability was investigated in 2P and MB strains serial crosses, the prsc mean (PM), the prsc midparent (MP) values (Ridley 2011) and the prsc trait variances of F_1 and F_2 progeny were compared. The units for PM and MP are prsc bristles. The virgin 2PHT individual of 2P strain was single paired with the opposed-sex virgin individual of 6, 8, 10, and 12PHT (from MB strain), while the virgin 12PHT individual was single paired with the oppose-sex virgin individual of 6, 8, 10, and 12PHT (all from MB strain). Each pairing test has 4–6 replicates, and the F_1 progeny was sibling mated to the F_2 generation for the prsc phenotype frequency distribution (PFD) analysis. The PM correlated to MP is illustrated in the PM–MP map (modified from the genotype–phenotype map, G–P map, Hansen 2006).

The cumulative effect of prsc variability changes

The F_1 sibling of 2P × MB cross was backcrossed to 2P and MB strains to examine the heritability of the prsc number variation. The resulted phenotypic traits were analyzed for the PM reduction from expected MP (Conner & Hartl 2004). PM reduction = 100% × 2 × (MP – PM)/(HPN – LPN), HPN and LPN are high and low parental prsc numbers in a cross.

The reproductive advantages between 2P and MB strains

The MB strain is essentially absented in the field population, and it is interesting to know whether the wild type (2P strain) has any reproductive advantage over MB strain. To simulate the field condition, a sub-population of 80 flies was created by grouping 20 virgin males, 20 virgin females from both 2P and MB strains in one cage to allow random mating occur, and the experiment was done in three replicates. The component analysis of prsc trait is possible, because the prsc traits of 2P and MB strains were completely independent from each other, while 94% prsc trait of F_1 progeny were independent with only 2.5% and 3.5% overlapping with respect to 2P and MB traits. In analysis, the overlapped phenotype frequencies were distributed by the peak height ratio to three trait standards.

Trend study of prsc variation

The F_1 offsprings of the random mating were sibling mated for three more generations, and their prsc PFDs were traced for variation.

RESULTS

The prsc variability was reduced by 2P strain

In 2P serial crosses, the PM of both F_1 and F_2 progeny were suppressed below 5 prsc, and lower than expected MP. The 2 × 12 cross for example, the PM = 4.7 reduced 46% from expected MP of 7.0 for both F_1 and F_2 progeny (Table 1 & Fig. 1A). In a 4–7 prsc MP window, the PM merely varied 1.1 prsc from 3.6 to 4.7 prsc and 3.5 to 4.7 prsc in F_1 and F_2 progeny, respectively (Table 1). The variability reduction or the biological buffering of prsc is illustrated in the PM–MP map (Fig. 2A). In MB serial crosses, the PM remained equally to MP in a 9–12 prsc window (Table 2), and followed the additive variation of quantitative genetics. The PM–MP map shows no sign of biological buffering (Fig. 2B). The variances of F_1 progeny in 2P series remained consistently at 1.0 (Table 1) and indifferent to the MP change. The variances of F_2 progeny increased with the MP (Table 1), but the 2PHT surge (Cheng *et al*. 2018) disturbed the continuous trait and

Table 1. The mean, variances, and midparent values of prescutellar bristles (prsc) in F1 and F2 progeny of the 2 prsc (2P) strain crosses.

		F_1		F_2	
Parent's phenotype	MP[z]	PM[y]	Var.[x]	PM	Var.
2 × 6	4.0	3.6 (513)[w]	1.0	3.5 (2,424)	2.0
2 × 8	5.0	4.3 (1,047)	1.1	4.0 (1,018)	2.6
2 × 10	6.0	4.7 (549)	1.1	4.4 (2,773)	4.3
2 × 12	7.0	4.7 (1,646)	0.9	4.7 (10,958)	3.7

[z] MP: midparent value.
[y] PM: prsc mean.
[x] Var.: variance of phenotype frequency distribution.
[w] Sample size.

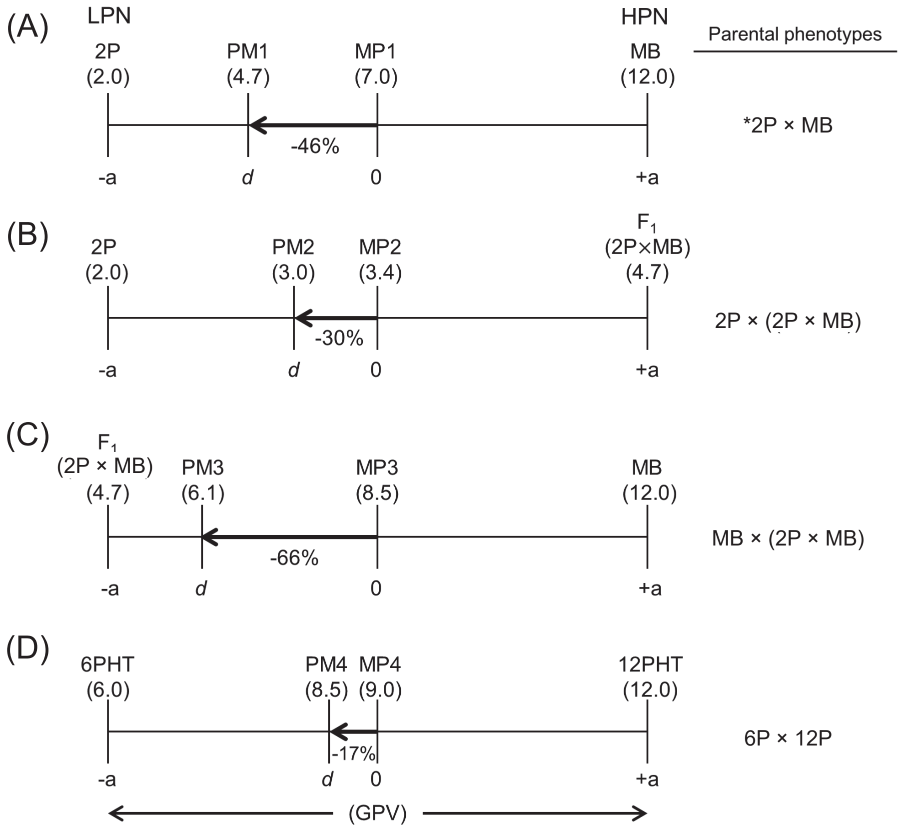

Fig. 1. The prescutellar bristles (prsc) mean (PM)/midparent (MP) shifts in two backcrosses (B & C). The horizontal scale represents genetic related phenotypic values (GPV). (A) (7.0 − 4.7) × 2 ÷ (12.0 − 2.0) = 46%; (B) (3.4 − 3.0) × 2 ÷ (4.7 − 2.0) = 30%; (C) (8.5 − 6.1) × 2 ÷ (12.0 − 4.7) = 66%; (D) (9.0 − 8.5) × 2 ÷ (12.0 − 6.0) = 17%. HPN: high parental prsc number in cross; LPN: low parental prsc number in cross. $a = (HPN - LPN)/2$; $d = (MP - PM)$; $d/a = 2 \times (MP - PM)/(HPN - LPN)$. *MB: 12 prsc phenotype; 2P: 2 prsc phenotype.

contributed part of variance increases. The variances of the MB series were positively correlated to the increases of MP, as $y = 6.34 \times ln(x) - 0.47$ ($R^2 = 0.84$) and $y = 9.03 \times ln(x) - 16.45$ ($R^2 = 0.91$) in F_1 and F_2 progeny, respectively.

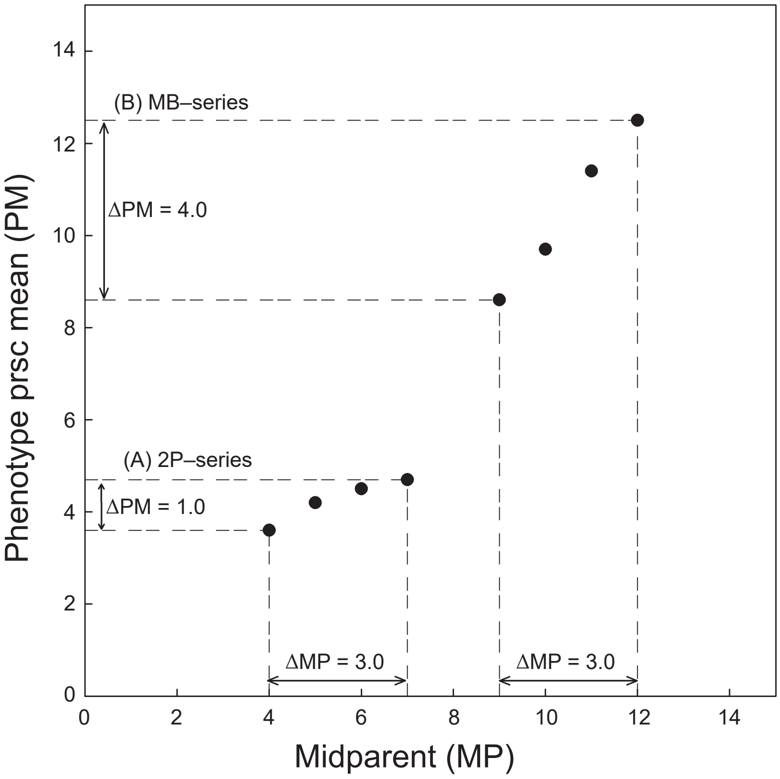

Fig. 2. The prescutellar bristles (prsc) mean (PM)–midparent (MP) map of 2 prsc (2P)– and multiple prsc (MB)–serial crosses. The mapping from MP to PM is a mathematical function that assigns a phenotypic change to each change in MP. (A) In 2P–series, the flat region a given MP change (ΔMP) has a small effect (ΔPM), whereas the same MP change in the steep region of the (B) MB–series has a large effect. Canalization is evolution toward flat regions, whereas decanalization is evolution toward steep regions (Hansen 2006).

Table 2. The mean, variances, and midparent value of prescutellar bristles (prsc) in F_1 and F_2 progeny of the multiple prsc (MB) strain crosses.

		F_1		F_2	
Parent's phenotype	MP[z]	PM[y]	Var.[x]	PM	Var.
12 × 6	9.0	8.5 (581)[w]	4.0	8.7 (992)	3.7
12 × 8	10.0	10.2 (601)	4.6	9.2 (783)	5.0
12 × 10	11.0	11.5 (220)	4.4	11.3 (946)	4.9
12 × 12	12.0	12.3 (3,338)	5.2	12.6 (3,872)	5.8

[z] MP: midparent value.
[y] PM: prsc mean.
[x] Var.: variance of phenotype frequency distribution.
[w] Sample size.

The prsc number reduction by 2P strain is cumulative and heritable

The 2P strain has been identified as the source of prsc variability reduction. In order to understand whether this reduction ability is heritable or not, the F_1 progeny of 2P × MB (Fig. 1A) was backcrossed to both 2P and MB strains. In 2P × (2P × MB) backcross (Fig. 1B), half the F_2 progeny were affected by

2PHT surge (Cheng *et al*. 2018), and after the correction, the MP was expected at 3.4. The PM of the backcross progeny was 3.0, a 30% reduction from the MP. The result indicated the prsc number reduction could be accumulated in repeated crosses of 2P strain. In MB × (2P × MB) backcross (Fig. 1C), the resulted PM was 6.1, a 66% reduction from the MP of 8.5. This second backcross confirmed the F_1 progeny still had the ability in reducing the prsc numbers. When contrasted to a comparable 12 × 6 cross in MB series (Fig. 1D), the progeny PM was 8.5, merely a 17% reduction from the MP of 9.0. The biological buffering effect continued in generations of 2P progeny, but not in a comparable cross of MB strain, suggesting that the genetic origin of prsc number reduction was related to the 2P strain and heritable.

The reproductive advantage of 2P strain in random mating with MB strain

In the grouping test, 2P × 2P, 2P × MB, and MB × MB are three possible combinations in random mating; each combination produces a unique progeny prsc trait with less than 2.5–3.5% overlapping (Fig. 3A). If the reproductive potential and the mating preference are equal, the probabilities of 2P individual to mate either 2P or MB opposed-sex individual were equal at 50%, and ideally, three components would be 25% : 50% : 25% or 1 : 2 : 1 for 2P, F_1, and MB traits, respectively (Russell 2005; Griffiths *et al*. 2008). The three distinct prsc phenotypic traits of random mating corresponded to 2P, MB, and F_1 trait standards, the overlapped areas were distributed according

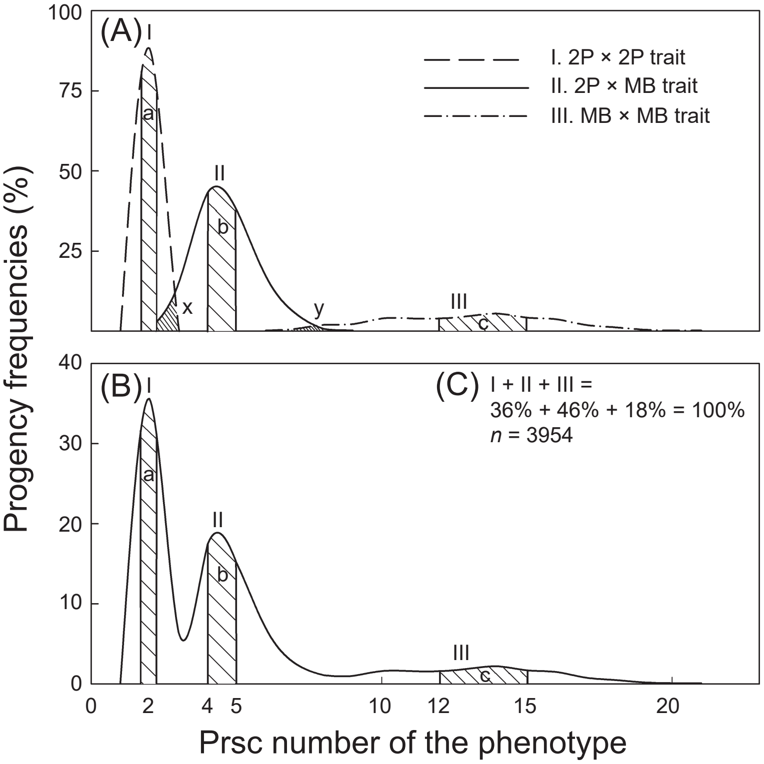

Fig. 3. The component analysis of progeny prescutellar bristles (prsc) traits in the grouping of 2 prsc (2P) and multiple prsc (MB) strains for random mating. (A) Three component traits with 2.5% overlapping (x) between (I) and (II) and 3.5% overlapping (y) between (II) and (III); (B) The overlapping was resolved by ratios between the peak areas (a, b & c, shade areas) to their own standards, a = 2PHT; b = 4–5PHT; c = 12–15PHT; and (C) The resulted progeny prsc traits and their component frequencies.

the peak ratio to their standards (Fig. 3B). The progeny frequencies were 36, 46 and 18% for 2P × 2P, 2P × MB and MB × MB mating combinations, respectively. The reproductive chances were 60% for the 2P individuals and 40% for the MB individuals, instead of expected 50%. The biological advantage of 2P strain is estimated at 3/2 over the MB strain.

The progeny of the grouping test

There were sibling mated for three more generations the PM reduced from 4.9 to 3.2, the variance lowered from 15.0 to 2.7, while the PFD range shortened from 2–19 to 2–11 (Fig. 4). The trend of prsc number reduction continued in these generations and the traits of MB strain gradually been eliminated as that in the field population.

DISCUSSION

In melon fly, the polygenic nature of prsc is contradictory to its "two phenotypes" expression in field population. How the continuous prsc trait reduced to two phenotypes is interesting to know. The paradox was only partially explained by a "2PHT surge" study (Cheng *et al*. 2018), because the inbreeding of F_1 is rare

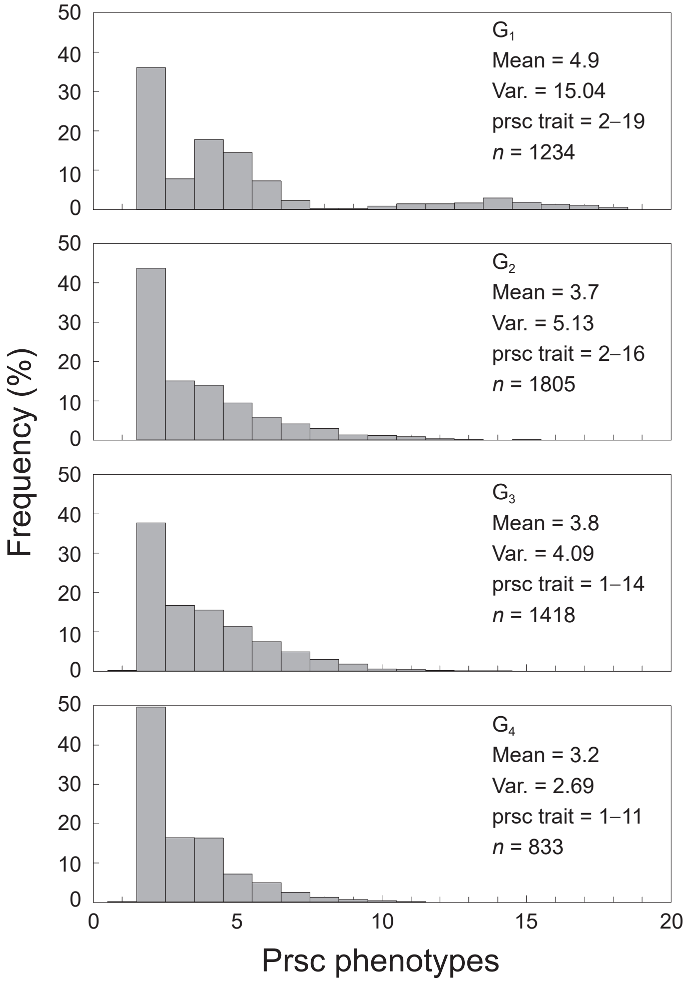

Fig. 4. The progeny prescutellar bristles (prsc) trait analysis in the grouping of 2 prsc (2P) and multiple prsc (MB) strains random mating for four generations.

in field, and the real reason for the wild type robustness is remain to be investigated. The robustness of 2P strain has been explored by comparing to a polygenic MB strain to show that 2P strain has the ability to suppress and buffer the prsc number in either single cross or multiple generations, and the reduction can be calculated (Conner & Hartl 2004).

The prsc variability reduction in this study corresponded to the definition of canalization of reduced trait variability and biological buffering (Waddington 1942; Gibson & Wagner 2000), so as the claim that the stabilizing selection will favor the wild type (Scharloo 1991). There is no previous report on the prsc genetics in melon fly, but the bristle number canalization has been recognized in *D. melanogaster* and related to chromosome 1 and/or 2 (canalizer) while chromosome 3 is responsible for the chaetogen, the embryonic cell to be developed into a bristle is increasing (Garcia-Vazquez & Rubio 1988). Wilkins (1986, 2002) stated that the canalization is by multiple genetic factors within the genome, a form of genetic buffering. The biological polymorphism provides subjects and materials for the trait variability study. Gibson & Hogness (1996) is able to show that canalization of the bithorax phenotype in Drosophila was largely caused by the polymorphism in the homeotic gene Ultrabithorax (Ubx).

This study observed two genetic modifications in prsc traits: (1) the 2P strain is capable in reducing the progeny's prsc number in cross with other phenotypes, and (2) the prsc variability reduction can be inherited and accumulated in following generations to continue the stabilizing selection. Both modifications contributed to the robustness of 2PHT and its domination in the field population as Slarkin (1985) indicated that the gene flow favoring the wild type to be continued and intensified for generations. Whether the prsc variability reduction fits the theoretical speculation of canalization (Wagner *et al*. 1997), in particular, the canalization proceeds in multiple generations or only in the embryo development remains to be discussed. Without the variability reduction, the prsc number in MB strain followed the additive variation of quantitative genetics accordingly. Since the MB strain has not been found in the field population and the Hardy-Weinberg equilibrium (Ridley 2011) between 2P and MB strains can never be an issue to be discussed. The reproductive advantage of 2P strain might be another factor in reducing or eliminating the MB traits in field population.

CONCLUSIONS

The prescutellar bristles of melon fly can be another polymorphism model, as *D. melanogaster*, to study the variability of quantitative traits. This prsc study began with a simple question of what is the prsc polymorphism in melon fly. First thing find out was the quantitative genetics of prsc, then came the genetic interaction of "2PHT surge" in a quantitative trait. The prsc variability reduction expended our view in wild type robustness and canalization. Now we know that the simple question asked in 1994 was not that simple. The genetics of prsc in melon fly is based on the quantitative genetics as the MB strain does; however, the 2P strain dominates the field population because it possess following characteristics in extra: (1) The 2P strain can reduce the prsc variability of MB strain in numbers as well as phenotypes. (2) The prsc variability reduction is heritable and cumulative, and maintains stabilizing selection in generations. (3) The reproductive advantage of 2P strain may also contribute the elimination of MB trait in field population.

REFERENCES

Cheng, E. Y., C. H. Wang, Y. B. Huang, and M. Y. Chiang. 2014. The quantitative genetics of prescutellar bristles in melon fly, *Bactrocera cucurbitae* (Coquillett). J. Taiwan Agric. Res. 63:179–187. (in Chinese with English abstract)

Cheng, E. Y., Z. H. Wang, Y. B. Huang, M. Y. Chiang, H. Y. Lu, D. H. Wu, C. M. Yang, and C. C. Nien. 2018. The genetic interaction on the quantitative traits of prescutellar bristles in melon fly, *Bactrocera* (*Zeugodacus*) *cucurbitae* (Coquillett). J. Taiwan Agric. Res. 67:9–15. (in Chinese with English abstract)

Conner, J. K. and D. L. Hartl. 2004. A Primer of Ecological Genetics. Sinauer Associates. Sunderland, MA. 304 pp.

Drew, R. A. I. and D. L. Hancock. 1994. Bulletin of Entomological Research Vol. 2. The *Bactrocera dorsalis* Complex of Fruit Flies (Diptera: Tephritidae: Dacinae) in Asia. CAB International. Wallinford, UK. 68 pp.

Emerson, R. A. and E. M. East. 1913. Research Bulletin. Vol. 2. The Inheritance of Quantitative Characters in Maize. University of Nebraska. Lincoln, NE. 120 pp.

Garcia-Bellido, A. 1979. Genetic analysis of the achaete-scute system of *Drosophila melanogaster*. Genetics 91:491–520.

Garcia-Vazquez, E. and J. Rubio. 1988. Chromosomal analysis of selected lines of *Drosophila melanogaster* with a new level of bristle canalization. Genet. Sel. Evol. 20:75–86.

Ghysen, A. and C. Dambly-Chaudière. 1988. From DNA to form: The achaete-scute complex. Genes Dev. 2:495–501.

Gibson, G. and D. S. Hogness. 1996. Effect of polymorphism in the *Drosophila* regulatory gene *Ultrabithorax* on homeotic stability. Science 271:200–203.

Gibson, G. and G. Wagner. 2000. Canalization in evolutionary genetics: A stabilizing theory? BioEssays 22:372–380.

Griffiths, A. J. F., S. R. Wessler, R. C. Lewontin, and S. B. Carroll. 2008. Introduction to Genetic Analysis. 9th ed. W. H. Freeman and Company. New York. 800 pp.

Hansen, T. F. 2006. The evolution of genetic architecture. Annu. Rev. Ecol. Evol. Syst. 37:123–157.

Lindsely, D. L. and G. G. Zimm. 1992. The Genome of *Drosophila melanogaster*. Academic Press. San Diego, CA. 1133 pp.

Ridley, M. 2011. Evolution. 3rd ed. Blackwell. Malden, MA. 751 pp.

Russell, P. J. 2005. iGenetics: A Mendelian Approach. Benjamin Cummings. San Francisco, CA. 842 pp.

Scharloo, W. 1991. Canalization: Genetic and developmental aspects. Annu. Rev. Ecol. Syst. 22:65–93.

Simpson, P., R. Woehl, and K. Usui. 1999. The development and evolution of bristle patterns in Diptera. Development 126:1349–1364.

Simpson, P. and S. Marcellini. 2006. The origin and evolution of stereotyped patterns of macrochaetes on the nota of cyclorraphous Diptera. Heredity 97:148–156.

Slarkin, M. 1985. Gene flow in natural populations. Annu. Rev. Ecol. Syst. 16:393–430.

Waddington, C. H. 1942. Canalization of development and the inheritance of acquired characters. Nature 150:563–565.

Waddington, C. H. 1947. Organisers and Genes. Cambridge University Press. Cambridge, UK. 160 pp.

Wagner, G. P., G. Booth, and H. Bagheri-Chaichian. 1997. A population genetic theory of canalization. Evolution 51:329–347.

White, I. M. and M. M. Elson-Harris. 1992. Fruit flies of economic significance: Their identification and bionomics. CAB International. Wallinford, UK. 601 pp.

Wilkins, A. S. 1986. Genetic Analysis of Animal Development. John Wiley & Sons. New York. 546 pp.

Wilkins, A. S. 2002. The Evolution of Developmental Pathways. Sinauer Associates. Sunderland, MA. 603 pp.

瓜實蠅 [*Bactrocera* (*Zeugodacus*) *cucurbitae* (Coquillett)] 中胸背板剛毛遺傳變異之縮減

鄭允[1] 王志賢[2] 黃毓斌[3,*] 江明耀[3] 呂秀英[4] 吳東鴻[5] 楊崇民[2] 粘君琪[2]

摘要

鄭允、王志賢、黃毓斌、江明耀、呂秀英、吳東鴻、楊崇民、粘君琪。2018。瓜實蠅 [*Bactrocera* (*Zeugodacus*) *cucurbitae* (Coquillett)] 中胸背板剛毛遺傳變異之縮減。台灣農業研究 67(4):337–346。

瓜實蠅 [*Bactrocera* (*Zeugodacus*) *cucurbitae* (Coquillett)] 之中胸背板剛毛 (prescutellar bristles; prsc) 係由數量遺傳 (quantitative genetics) 控制，其數量由 2–20 根不等，但野生族群中，2 根剛毛之表現型 (2 prsc phenotype; 2PHT) 卻占 95%，而偏離了連續分布 (continuous trait) 之基本分布，其原因究竟為何？本研究是以 2 prsc (2P) 品系與多剛毛品系 (multiple prsc strain; MB-strain) 進行雜交並觀察其子代之表現型分布頻度進行分析，觀察結果發現 2P 品系 (野生型) 為親本時，壓縮多剛毛品系之表現而偏離數量遺傳之均值原則 (additive)，在二者之子代中，親本之均值 (midparent; MP) 變化為 4–8 prsc 間時，子代 prsc mean (PM) 值為 3.6–4.7 其範圍僅為 1.1，有明顯的緩衝現象 (biological buffering)，可在 MP/PM 圖示中顯示 (比照 genotype–phenotype map, Hansen 2006)。多剛毛品系不同表現型之子代 prsc 則與親本之平均值成正比。MP 在 9–12 prsc 間，PM 之變異也為 9–12 prsc，而依數量遺傳之 additive 準則互動。依 Conner & Hartl (2004) 報導，就 PM 偏離均質 (MP) 方法分析，發現 2P 品系後代之 PM 均低於 MP，在二則回交測試中均顯示 2P 品系之 PM 壓縮特質為可遺傳性，在後繼之子代中且有累進壓縮 prsc 之效應。而在模擬 2P 及 MB 兩品系，在田間以群體方式進行自由選擇繁殖 (random mating) 所產生之子代表現型頻度，發現 2P 品系對 MB 品系有 3 : 2 之繁殖優勢，而其後續繁殖之子代中，中胸剛毛之平均數量及分布均不斷地減縮，與觀察田間族群之低變異性所得相符。由此研究所得結論為 2P 品系有減少子代 prsc 數量之特質，此特質有遺傳性及累進性。只要與 2P 品系雜交後，prsc 數量即不斷地減少，而趨向田間所見之 2P 野生型。

關鍵詞：數量遺傳、中胸背板剛毛、瓜實蠅。

投稿日期：2017 年 7 月 19 日；接受日期：2018 年 5 月 30 日。

* 通訊作者：ybhuang@tari.gov.tw
[1] 農委會農業試驗所應用動物組前研究員。台灣 台中市。
[2] 農委會農業試驗所應用動物組技工。台灣 台中市。
[3] 農委會農業試驗所應用動物組助理研究員。台灣 台中市。
[4] 農委會苗栗區農業改良場場長。台灣 苗栗縣。
[5] 農委會農業試驗所作物組助理研究員。台灣 台中市。

台灣農業研究 (J. Taiwan Agric. Res.) 67(4):347–354 (2018)
DOI:10.6156/JTAR.201812_67(4).0003 研究報告

番椒微斑病毒 (*Pepper mild mottle virus*) 田間發生與器械消毒管理

曹麗玉 [1,*]

摘要

曹麗玉。2018。番椒微斑病毒 (*Pepper mild mottle virus*) 田間發生與器械消毒管理。台灣農業研究 67(4):347–354。

應用間接酵素連結免疫分析法 (indirect enzyme-link immunosorbent assay; ELISA) 進行 9 種不同病毒：番椒黃化病毒 (*Capsicum chlorosis virus*; CaCV)、辣椒葉脈斑駁病毒 (*Chilli veinal mottle virus*; ChiVMV)、胡瓜嵌紋病毒 (*Cucumber mosaic virus*; CMV)、番椒葉脈斑駁病毒 (*Pepper veinal mottle virus*; PVMV)、番椒微斑病毒 (*Pepper mild mottle virus*; PMMoV)、馬鈴薯 Y 病毒 (*Potato virus Y*; PVY)、番茄斑萎病毒 (*Tomato spotted wilt virus*; TSWV)、煙草嵌紋病毒 (*Tobacco mosaic virus*; TMV)、番茄嵌紋病毒 (*Tomato mosaic virus*; ToMV) 的血清學檢測，定期調查高雄、屏東地區之甜椒簡易網室設施及露天栽培園之嵌紋型病毒病害發生種類。結果顯示，簡易網室及露天栽培之甜椒均以 PVMY、ChiVMV、PVY 及 PMMoV 4 種病毒感染為主，而露天栽培園之病毒感染情形明顯較簡易網室栽培嚴重。病毒複合感染對於植株造成的病徵比單一病毒感染之病徵嚴重，單獨感染多出現輕微的斑駁嵌紋，複合感染易造成葉片及果實嚴重畸型，造成植株矮化及生長停滯。PMMoV 可經由種子傳播，以種子直接研磨及種子直播穴盤可測出種子之病毒帶毒率。目前已檢測 20 批甜椒種子的帶毒率，其中綠色甜椒「群星」、「藍星」及彩色甜椒「北極星」各一批號測得 PMMoV 感染，病毒感染率分別為 13、18 及 17%。將人工汙染 PMMoV 病種子置於細網袋 (100 粒/袋)，分別以 12.5、15 及 20% 的磷酸三鈉溶液浸泡 10 min、20 min、30 min 後，以活水沖洗約 60 min，以 1% 次氯酸鈉去除種子表面雜菌，置於濕濾紙上使之萌芽，調查種苗發芽率及病毒帶毒率。其中，以 12.5% 磷酸三鈉溶液處理 30 min (病毒帶毒率 5%)、15% 磷酸三鈉溶液處理 30 min (病毒帶毒率 3%) 及 20% 磷酸三鈉溶液處理 20 min (病毒帶毒率 5%) 之去病毒能力最好。大部分病毒可經由機械方式感染其他植株，在器械消毒試驗方面，以 5% 次氯酸鈉及 0.5% 次氯酸鈉在 3 次試驗中的去毒效果均可達 95% 以上，20% 脫脂奶粉去毒效果次之。

關鍵詞：番椒微斑病毒、器械消毒、種子消毒。

前言

番椒屬茄科 (*Solanaceae*) 番椒屬 (*Capsicum*)，其中具辛辣味者稱為辣椒 (hot pepper)，而不具辛辣味者稱為甜椒 (sweet pepper)，統稱為番椒 (pepper)，學名為 *Capsicum annuum* L.。番椒原產地為中美洲、南美洲熱帶地區。哥倫布於 1493 年首先帶回西班牙，於 15 世紀中葉於歐洲大陸流傳，16 世紀時傳至日本，17 世紀始傳到中國。台灣甜椒栽培始由美國於 1960 年之後引進栽培，週年均有生產，夏季多種植於中海拔地區，秋冬季則以中、南部地區栽培較多。依據 2016 年台灣農業統計年報資料記載，台灣地區全年作甜椒種植面積約為 1,255.7 ha，年產量約 17,323 Mg，其中以中部的南投縣、雲林縣、嘉義縣及南部的屏東縣為主要栽培區，分別占全國栽培面積的 28.7、

投稿日期：2017 年 10 月 20 日；接受日期：2018 年 5 月 14 日。
[*] 通訊作者：tsly@fthes-tari.gov.tw
[1] 農委會農業試驗所鳳山熱帶園藝試驗分所植物保護系助理研究員。台灣 高雄市。

20.2、18.5 及 12.3%。

台灣地區甜椒常見之病害如：病毒病、青枯病、細菌性斑點病、疫病、白粉病、炭疽病、菌核病等，其中甜椒的病毒病害根據國外文獻記載有 20 種以上 (Green & Kim 1991; Agranovsky 1993; Luis-Arteaga & Ponz 2003; Kenyon *et al*. 2014; Jo *et al*. 2017)。根據台灣植物病害名彙及相關研究，可感染甜椒的病毒種類有辣椒葉脈斑駁病毒 (*Chilli veinal mottle virus*; ChiVMV)、番椒葉脈斑駁病毒 (*Pepper veinal mottle virus*; PVMV)、馬鈴薯 Y 病毒 (*Potato virus Y*; PVY)、番椒黃化病毒 (*Capsicum chlorosis virus*; CaCV)、番茄斑萎病毒 (*Tomato spotted wilt virus*; TSWV)、胡瓜嵌紋病毒 (*Cucumber mosaic virus*; CMV)、番茄黃化捲葉病毒 (*Tomato yellow leaf curl virus*; TYLCV)、煙草捲葉病毒 (*Tobacco leaf curl virus*; TLCV)、甜椒微斑病毒 (*Pepper mild mottle virus*; PMMoV)、煙草嵌紋病毒 (*Tobacco mosaic virus*; TMV) 及番茄嵌紋病毒 (*Tomato mosaic virus*; ToMV) 等 11 種。上述病毒中，同屬於 *Tobamovirus* 病毒群的 TMV、ToMV、PMMoV 經由機械傳播外，其餘病毒均可經由媒介昆蟲傳播。其中， CMV (屬於 *Cucumovirus* 屬)、PVY、PVMV、ChiVMV (三者同屬於 *Potyvirus* 屬) 可經由蚜蟲傳播，TSMV 及 CaCV (二者同屬於 *Tospovirus* 屬) 經由薊馬傳播，TYLCV 及 TLCV (二者同屬於 *Begomovirus* 屬) 經由銀葉粉蝨傳播 (Zaitlin & Israel 1975; Broadbent 1976; Wetter & Conti 1988; Green & Kim 1991; Lin *et al*. 2005; Moury *et al*. 2005; Cheng *et al*. 2009; Shih *et al*. 2010; Yoshimoto *et al*. 2012; Cheng *et al*. 2013; Kenyon *et al*. 2014)。

PMMoV 為種傳病毒，對番椒的生產影響很大，除了造成果實的外觀產生畸型或變小，使得產品失去商業價值外，對產量影響也很大 (Wetter & Conti 1988; Adkins *et al*. 2001; Genda *et al*. 2005)。此類 *Tobamovirus* 病毒在環境中存活能力相當強，且容易經由機械方式感染其他植株，造成連作時的主要病害 (Scholthof 2004)。因此，在田間栽培時，手部及工作器械的消毒可降低病毒感染。有些消毒方法，如：利用脫脂奶粉防治瓜類白粉病 (Bettiol 1999; Bettiol *et al*. 2008)、利用牛奶防治煙草嵌紋病毒 (Apple *et al*. 1963)，或漂白水消毒法 (Broadbent & Fletcher 1963; Broadbent 1976; Sánchez-Navarro *et al*. 2007; Lewandowski *et al*. 2010; Li *et al*. 2015) 皆有進一步確認及改進的空間。本試驗擬研究在現有田間栽培模式下，PMMoV 的自然傳播方式，比較幾種常用的消毒藥劑 (如：次氯酸鈉、磷酸三鈉鹽、酒精、脫脂奶粉等) 進行器械及手部消毒，降低田間病毒感染率。除確認坊間的消毒方法外，希望能從日常生活中尋求有效且易取得之消毒劑供農民在生產時使用，並建立田間標準操作方式以生產低帶毒種子及種苗，以提供種苗生產及農民參考使用。

材料與方法

調查甜椒病毒病害發生種類

採樣：調查南部地區 (高雄、屏東) 之簡易網室設施及露天栽培甜椒園之病毒病害發生種類，每園先以目視方式隨機調查 200 株，記錄有疑似病毒感染株數，並採集疑似病毒感染株葉片回到實驗室，用 9 種不同病毒 (CaCV、ChiVMV、CMV、PVMV、PMMoV、PVY、TSWV、TMV、ToMV) 抗血清以間接酵素連結免疫分析法 (indirect enzyme-link immunosorbent assay; ELISA) 檢測。

病毒抗血清來源：CaCV、ChiVMV、PVMV、PMMoV、PVY、TSWV、ToMV 係購自 Agdia® (Elkhart, IN, USA)，CMV、TMV 取自實驗室自行製備。

間接酵素連結免疫分析法：秤取葉片樣品 0.3 g，以 3 mL 之 15 mM 碳酸鈉緩衝液 (sodium carbonate buffer, pH 9.6) 研磨均勻後靜置。上澄液加入 ELISA 反應盤內，每個樣品 2 重複，置於 37℃ 定溫箱進行 4 h 的覆膜反應 (coating reaction)。其後，以 1× PBST (phosphate buffered saline with tween-20) 緩衝液 (137 mM NaCl, 1.5 mM KH_2PO_4, 1 mM Na_2HPO_4, 0.05% Tween 20, pH 7.4) 沖洗 3 次；加入病毒抗血清後置於 37℃ 定溫箱反應 2 h；以 1× PBST 緩衝液沖洗 3 次後，加入以 1× PBST 緩衝液稀釋的黏附鹼

性磷酸酵素之山羊抗兔二次抗體 (Alkaline Phsphatase-conjugated AffiniPure Goat anti-Rabbit immunoglobin, Jackson, West Grove, PA, USA) 置於 37℃定溫箱反應 2 h；以 1× PBST 緩衝液沖洗 3 次後，最後加入濃度為 1mg mL^{-1} 之鹼性磷酸酵素反應基質 (p-nitrophenyl phosphate, Sigma-Aldrich, St. Louis, MS, USA) 進行呈色反應。反應 30–60 min，以 ELISA 判讀儀 (Emax™ Microplate Reader, Molecular Devices, Sunnyvale, CA, USA) 讀取 OD_{405nm} 之吸收值，作為評估病毒濃度高低之依據。樣品讀值大於健康葉片之 2 倍者，視為正反應。

檢測甜椒種子 PMMoV 帶毒率

收集市售甜椒品種 (系或批號) 10 種以上。每批種子隨機檢測 1,000 粒種子 (每 10 粒為一個樣品)，樣品先加入液態氮研磨後，再以 PMMoV 抗血清及上述間接 ELISA 進行檢測，計算各批種子之帶毒率。

PMMoV 帶毒器械獲得方式

以徒手沾取 PMMoV 病組織研磨液 (病組織採自接種 PMMoV 之圓葉煙草 *Nicotiana benthamiana*)，每克罹病葉片加入 10 mL 100 mM 磷酸鈉緩衝液 (100 mM Na_2HPO_4, 100 mM NaH_2PO_4, pH 7.2)，塗抹健康植株或以剪刀沾取病組織研磨液修剪健株後，以目視觀察病徵計算傳毒比率並以 ELISA 確認。

操作器械消毒試驗

仿效坊間以手部及修剪器械修剪枝條摘心整蔓，使用沾有 PMMoV 病組織汁液的工具及手進行修剪，惟工具及手均先充分浸泡 (或搓洗) 於 10%、20% 脫脂奶粉 (Anchor®, Auckland, New Zealand) 溶液，分別處理不同時間 (分別浸泡 10 s、20 s、30 s 至 1 min)，再用清水沖洗或浸泡後再行修剪，以目視病徵及 ELISA 評估消毒效果。

操作器械消毒試驗—化學消毒藥劑消毒試驗

測試化學藥劑消毒之最佳稀釋度。配製不同稀釋濃度 (0.5%、5%、10%) 之次氯酸鈉 (hypochloride, Chlorax®, Oakland, CA, USA)、不同稀釋濃度 (75%、100%) 酒精 (absolute alcohol, Sigma-Aldrich, St. Louis, MS, USA) 溶液備用。沾有 PMMoV 病組織汁液的工具均先分別浸泡於上述溶液，並分別處理不同時間 (分別浸泡 10 s、20 s、30 s 至 1 min)，再用清水沖洗或浸泡後進行修剪，以目視病徵及 ELISA 評估消毒效果。

種子消毒試驗

將以人工污染的 PMMoV 病種子置於細網袋 (每袋 100 粒)，分別以 12.5、15 及 20% 的磷酸三鈉 (Trisodium phosphate, Sigma-Aldrich, St. Louis, MS, USA) 溶液浸泡 10 min、20 min、30 min 後，以活水沖洗約 60 min。以 1% 次氯酸鈉去除種子表面雜菌，置於濕濾紙上使之萌芽，調查種苗發芽率及病毒帶毒率。

結果

調查甜椒病毒病害發生種類

調查 2015 年南部地區 (高雄、屏東) 之甜椒簡易網室設施及露天栽培園之病毒病害發生種類，採集疑似病毒感染植株葉片以 9 種不同病毒 (CaCV、ChiVMV、CMV、PVMV、PMMoV、PVY、TSWV、TMV、ToMV) 抗血清以間接 ELISA 分析法檢測，檢測的病毒中以 ChiVMV 感染率最高 (25–30%)、PVMV 感染率次之 (22–27%) (表 1)。1–5 月及 8–12 月簡易網室及露天栽培之甜椒，均以 PVMY、ChiVMV、PVY 及 PMMoV 感染為主，而露天栽培園之病毒感染情形明顯較簡易網室栽培嚴重 (圖 1)。病毒單獨感染多出現輕微的斑駁嵌紋，複合感染易造成葉片及果實嚴重畸型或頂芽壞疽，造成植株矮化及生長停滯。

檢測種子帶毒 (PMMoV) 率

PMMoV 可經由種子傳播，故將種子以液態氮直接研磨 (每批種子隨機檢測 1,000 粒種子，每 10 粒為一個樣品) 調查各批種子之 PMMoV 病毒帶毒率。目前已檢測綠色甜椒「群

表 1. 2015 年高屏地區甜椒嵌紋型病毒病害發生調查。

Table 1. Investigation of the virus diseases on sweet pepper from Kaohsiung and Pintung areas in 2015.

Virus incidence[z]	Location area			
	Pintung	Yanpu	Fengshan	Luzhu
ChiVMV	25	28	30	27
PVMV	27	25	27	22
PVY	18	21	15	18
PMMoV	14	17	13	12
CMV	8	10	10	7
TMV、ToMV	8	5	12	10
TSWV、CaCV	3	7	4	7

[z] Percentage of virus infection. ChiVMV: *Chilli veinal mottle virus*; PVMV: *Pepper veinal mottle virus*; PVY: *Potato virus Y*; PMMoV: *Pepper mild mottle virus*. CMV: *Cucumber mosaic virus*; TMV: *Tobacco mosaic virus*; ToMV: *Tomato mosaic virus*; TSWV: *Tomato spotted wilt virus*; and CaCV: *Capsicum chlorosis virus*.

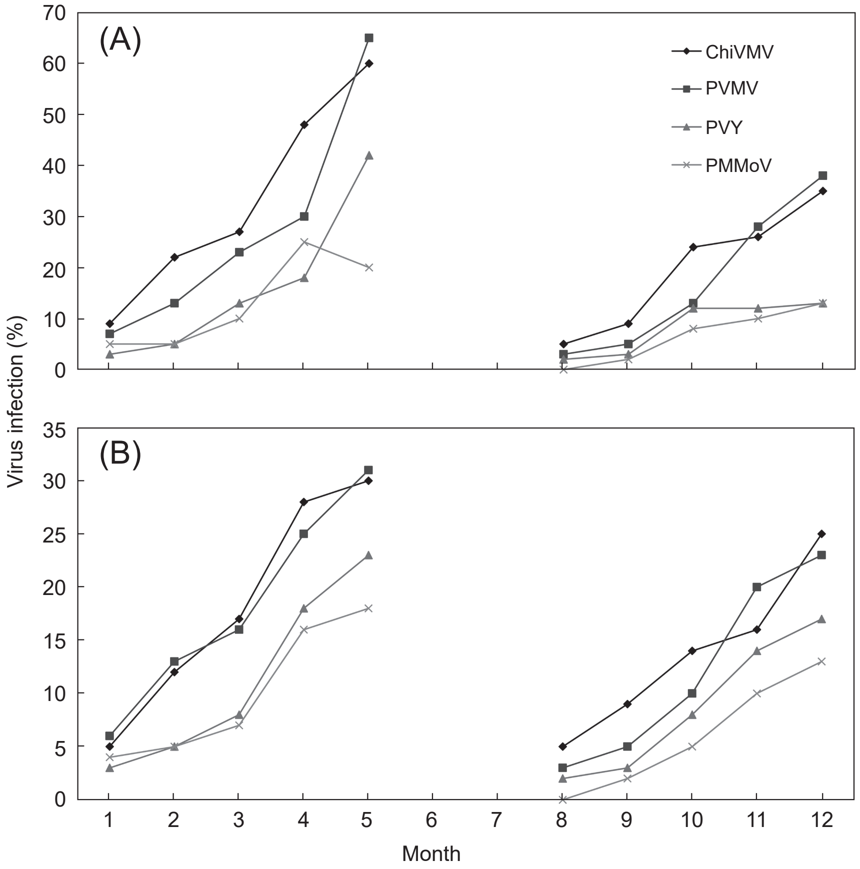

圖 1. 2015 年 1–12 月 4 種病毒於 (A) 露天栽培甜椒園 (B) 簡易網室甜椒園之感染情形。

Fig. 1. Investigation of the 4 virus diseases on pepper in (A) open fields and (B) net-greenhouses from January to December in 2015. ChiVMV: *Chilli veinal mottle virus*; PVMV: *Pepper veinal mottle virus*; PVY: *Potato virus Y*; and PMMoV: *Pepper mild mottle virus*.

星」、「女王星」、「藍星」各4批號及彩色甜椒「北極星」、「麗妃星」、「金明星」各3批號的種子帶毒率調查，其中綠色甜椒「群星」、「藍星」及彩色甜椒「北極星」各1批號測得 PMMoV 感染，病毒感染率分別為 63、78 及 62%。此 3 批帶有 PMMoV 種子播入穴盤 (每批種子隨機直播 100 粒種子)，待長出 3 片真葉後以 PMMoV 抗血清進行檢測，PMMoV 檢出率為 13、18 及 17%。

操作器械消毒試驗

有些病毒可經由機械方式造成的傷口感染其他植株，因此在田間栽培時，注意手部及器械的消毒可降低病毒傳播的速率。在本次器械消毒試驗，測試 20% 及 10% 脫脂奶粉、市售家用漂白水 (5% 次氯酸鈉、0.5% 次氯酸鈉)、酒精 (95%、75%) 及磷酸三鈉鹽對於修剪工具去毒效果，其中以 5% 次氯酸鈉及 0.5% 次氯酸鈉在 3 次試驗中的去毒效果均可達 95% 以上，20% 脫脂奶粉去毒效果次之 (表 2)。

種子消毒試驗

將人工污染的 PMMoV 病種子置於細網袋，以 3 種不同濃度的磷酸三鈉溶液分別浸泡 10 min、20 min、30 min 後，以活水沖洗約 60 min，經過 1% 次氯酸納去除種子表面雜菌，種子發芽率均可維持在 80% 以上。在 3 次的種子消毒試驗中，結果顯示以 12.5% 磷酸三鈉溶液處理 30 min (平均病毒帶毒率 5%)、15% 磷酸三鈉溶液處理 30 min (平均病毒帶毒率 3%) 及 20% 磷酸三鈉溶液處理 20 min (平均病毒帶毒率 5%) 之去病毒能力最好 (表 3)。

討論

台灣地區的甜椒栽培方式有設施栽培和露天栽培，檢視甜椒隨機採集樣本來源，樣本採集自設施栽培甜椒者，病毒發病率介於 2–10% 之間；樣本採集自露天栽培田區，病毒發病率差異很大，介於 52–85%。以 PVMY、ChiVMV 及 PVY、PMMoV 感染為主。甜椒病毒病害發生調查的檢測樣本中，PMMoV 多與 PVMV、ChiVMV 複合感染。甜椒於台灣多為露天栽培，彩色甜椒多以設施栽培為主，病毒感染植株常呈現局部集中發生而呈現塊狀分布，又甜椒為連續採收作物，且栽培過程需持續進行側芽摘除，故推測病毒可能經由去芽或採果所造成的傷口進行傳播。一植行中若有 1、2 株罹病，藉整枝摘芽就可迅速傳播開 (Broadbent 1963; Broadbent & Fletcher 1963; Wetter & Conti 1988; Green & Kim 1991)。

由於上述因素，本研究嘗試以常用消毒

表 2. 受 PMMoV 汙染剪刀 (或刀片) 浸泡不同消毒藥劑對於 PMMoV 之去毒效果。

Table 2. Incidence of *Pepper mild mottle virus* (PMMoV) infection on pepper plants resulting from preliminary disinfection screens using virus-contaminated razor knives or blades.

	Incidence of PMMoV infection		
Treatment[z]	I	II	III
20% NFDM (wt vol^{-1})	3/20 (15%)	2/20 (10%)	2/20 (10%)
10% NFDM (wt vol^{-1})	4/20 (20%)	4/20 (20%)	3/20 (15%)
5% NaOCl [Clorax® (1 : 1)]	0/20 (0%)	0/20 (0%)	0/20 (0%)
0.5% NaOCl [Clorax® (1 : 10)]	0/20 (0%)	1/20 (5%)	1/20 (5%)
95% Alcohol	4/20 (20%)	3/20 (15%)	3/20 (15%)
75% Alcohol	5/20 (25%)	4/20 (20%)	3/20 (15%)
3% TSP (wt vol^{-1})	5/20 (25%)	4/20 (20%)	3/20 (15%)
Fire (20 s)	0/20	0/20	0/20
Mock (water)	20/20 (100%)	20/20 (100%)	20/20 (100%)

[z] Each razor blade was contaminated for 30 s in sap from a PMMoV-infected pepper plant, briefly rinsed in deionized water, followed by a 1 min disinfection. NFDM: nonfat dry milk; TSP: trisodium phosphate.

表 3. PMMoV 罹病種子浸泡不同濃度及處理時間之磷酸三鈉對於 PMMoV 之去毒效果。

Table 3. Incidence of *Pepper mild mottle virus* (PMMoV) infection on pepper seeds resulting from preliminary disinfection screens using different concentrations and treatment times.

Treatment[z]	Incidence of PMMoV infection		
	I	II	III
12.5% TSP (10 min)	32%	37%	28%
(20 min)	21%	27%	23%
(30 min)	7%	5%	4%
15% TSP (10 min)	31%	34%	31%
(20 min)	23%	28%	27%
(30 min)	4%	3%	3%
20% TSP (10 min)	28%	32%	29%
(20 min)	5%	7%	3%
(30 min)	-	-	-
Mock (water)	88%	91%	85%

[z] PMMoV infected seeds were placed in a net bag (100 seeds per bag), soaked in 12.5%, 15%, and 20% trisodium phosphate solution for 10 min, 20 min, and 30 min, respectively. TSP: trisodium phosphate.

藥劑進行器械消毒試驗。次氯酸是很有效的殺毒藥劑 (稀釋濃度 0.5–10.0%)，可用於避免 *Tobamovirus* 經由受感染的修剪刀片傳播 (Kamenova & Adkins 2004; Lewandowski *et al*. 2010)。在本研究的修剪器械消毒試驗方面，以 Clorax 漂白水 (5% 次氯酸) 或 1：10 (0.5% 次氯酸) 浸泡 1 min 之去毒效果最好，20% 脫脂奶粉去毒效果次之。稀釋 10× 的家用漂白水 (0.5% 次氯酸鈉) 去毒效果雖好，但必需注意稀釋後要儘快使用 (不要超過 1 d)，以免影響消毒效果。20% 脫脂奶粉去除效果可達 85%，但使用過程中容易有沉澱分層的問題，未來使用時可添加 0.1% Tween 20 來改善 (Belliol 1999; Sánchez-Navarro *et al*. 2007; Lewandowski *et al*. 2010)。

由於 PMMoV 亦可經由帶毒種子進行病毒傳播，故調查市售甜椒種子 PMMoV 帶毒率。已完成綠色甜椒「群星」、「女王星」、「藍星」及彩色甜椒「北極星」、「麗妃星」、「金明星」共 20 批號的種子帶毒率調查，其中綠色甜椒「群星」、「藍星」及彩色甜椒「北極星」各一批號測得 PMMoV 感染，病毒感染率分別為 23、18 及 27%。

PMMoV 病種子以不同濃度的磷酸三鈉溶液浸泡處理，在 3 次的種子消毒試驗中，結果顯示以 12.5% 磷酸三鈉溶液處理 30 min (平均病毒帶毒率為 5%)、15% 磷酸三鈉溶液處理 30 min (平均病毒帶毒率 3%) 及 20% 磷酸三鈉溶液處理 20 min (平均病毒帶毒率 5%) 之去病毒能力最好，可提供種苗生產業者參考使用。並且，建議種苗生產公司與農民應建立無病毒種子篩檢制度，配合田間媒介昆蟲防治及其他肥培管理措施，應可有效降低本病之發生及其引起之農業損失。

引用文獻

Adkins, S., E. M. Lamb, P. D. Roberts, M. D. Gooch, L. Breman, and K. D. Shuler. 2001. Identification of *Pepper mild mottle virus* in commercial bell pepper in Florida. Plant Dis. 85:679.

Agranovsky, A. A. 1993. Virus diseases of pepper (*Capsicum annuum* L.) in Ethiopia. J. Phytopathol. 138:89–97.

Apple, J. L., J. F. Chaplin, and T. J. Mann. 1963. Comparison of the *Nicotiana glutinosa* resistance factor and milk in controlling mosaic in flue-cured tobacco. Tob. Sci. 7:46–53.

Bettiol, W. 1999. Effectiveness of cow's milk against zucchini squash powdery mildew (*Sphaerotheca fuliginea*) in greenhouse conditions. Crop Prot. 18:489–492.

Bettiol, W., H. S. A. Silva, and R. C. Reis. 2008. Effectiveness of whey against zucchini squash and cu-

cumber powdery mildew. Sci. Hortic. 117:82–84.

Broadbent, L. 1963. The epidemiology of Tomato mosaic III. Cleaning virus from hands and tools. Ann. Appl. Biol. 52:225–232.

Broadbent, L. 1976. Epidemiology and control of *Tomato mosaic virus*. Annu. Rev. Phytopathol. 14:75–96.

Broadbent, L. and J. T. Fletcher. 1963. The epidemiology of tomato mosaic IV. Persistence of virus on clothing and glasshouse structures. Ann. Appl. Biol. 52:233–241.

Cheng, Y. H., R. Y. Wang, C. C. Chen, C. A. Chang, and F. J. Tan. 2009. First report of *Pepper veinal mottle virus* in tomato and pepper in Taiwan. Plant Dis. 93:107.

Cheng, Y. H., T. C. Deng, C. C. Chen, C. P. Kuan, and C. A. Chang. 2013. Identification and occurrence of *Pepper mottle virus* isolated from peppers in Taiwan. J. Taiwan Agric. Res. 62:360–371. (in Chinese with English abstract)

Genda, Y., K. Sato, O. Nunomura, T. Hirabayashi, J. Ohnishi, and S. Tsuda. 2005. Immunolocalization of *Pepper mild mottle virus* in *Capsicum annuum* seeds. J. Gen. Plant Pathol. 71:238–242.

Green, S. K. and J. S. Kim. 1991. Characteristics and Control of Viruses Infecting Peppers: A Literature review. Asian Vegetable Research and Development Center. Taipei, Taiwan. 60 pp.

Jo, Y., H. Choi, S. M. Kim, S. L. Kim, B. C. Lee, and W. K. Cho. 2017. The pepper virome: Natural co-infection of diverse viruses and their quasispecies. BMC Genomics 18:453.

Kamenova, L. and S. Adkins. 2004. Transmission, in planta distribution, and management of *Hibiscus latent Fort Pierce virus*, a novel tobamovirus isolated from Florida hibiscus. Plant Dis. 88:674–679.

Kenyon, L., S. Kumar, W. S. Tsai, and J. D. Hughes. 2014. Virus diseases of peppers (*Capsicum* spp.) and their control. Adv. Virus Res. 90:297–354.

Lewandowski, D. J, A. J. Hayes, and S. Adkins. 2010. Surprising results from a search for effective disinfectants for *Tobacco mosaic virus*-contaminated tools. Plant Dis. 94:542–550.

Li, R., F. Baysal-Gurel, Z. Abdo, S. A. Miller, and K. S. Ling. 2015. Evaluation of disinfectants to prevent mechanical transmission of viruses and a viroid in greenhouse tomato production. Virol. J. 12:5.

Lin, F. C., T. T. Hsieh, and C. L. Wang. 2005. Occurrence of whiteflies and their integrated management in Taiwan. p.245–257. *in*: Proceedings of the International Seminar on Whitefly Management and Control Strategy. October 3–8, 2005. Taichung, Taiwan. Food and Fertilizer Technology Center and Agricultural Research Institute Press. Taichung, Taiwan.

Luis-Arteaga, M. and F. Ponz. 2003. Potato virus Y. p. 34–37. *in*: Compendium of Pepper Diseases. (Pernezny, K. L., P. D. Roberts, J. F. Murphy, and N. P. Goldberg, eds.) APS Press. Saint Paul, MN. 88 pp.

Moury, B., A. Palloix, C. Caranta, P. Gognalons, S. Souche, K. G. Selassie, and G. Marchoux. 2005. Serological, molecular, and pathotype diversity of *Pepper veinal mottle virus* and *Chili veinal mottle virus*. Phytopathology 95:227–232.

Sánchez-Navarro, J. A., M. C. Cañizares, E. A. Cano, and V. Pallása. 2007. Plant tissue distribution and chemical inactivation of six carnation viruses. Crop Prot. 26:1049–1054.

Scholthof, K. B. G. 2004. Tobacco mosaic virus: A model system for plant biology. Annu. Rev. Phytopathol. 42:13–34.

Shih, S. L., W. S. Tsai, L. M. Lee, J. T. Wang, S. K. Green, and L. Kenyon. 2010. First report of *Tomato yellow leaf curl Thailand virus* associated with pepper leaf curl disease in Taiwan. Plant Dis. 94:637.

Wetter, C. and M. Conti. 1988. *Pepper mild mottle virus*. CMI/AAB Descriptions of Plant Viruses No. 330. Commonwealth Mycological Institute. Kew, UK. http://www.dpvweb.net/dpv/showdpv.php?dpvno=330 (visit on 3/17/2010)

Yoshimoto, R., H. Sasaki, T. Takahashi, H. Kanno, and M. Nanzyo. 2012. Contribution of soil components to adsorption of *Pepper mild mottle virus* by Japanese soils. Soil Biol. Biochem. 46:96–102.

Zaitlin, M. and H. W. Israel. 1975. *Tobacco mosaic virus* (type strain). CMI/AAB Descriptions of Plant Viruse: No. 151. Commonwealth Mycological Institute. Kew, UK. http://www.dpvweb.net/dpv/showdpv.php?dpvno=151 (visit on 12/15/2008)

Investigation of *Pepper mild mottle virus* on Peppers and the Disinfection of Cutting Tools in Taiwan

Li-Yu Tsao[1,*]

Abstract

Tsao, L. Y. 2018. Investigation of *Pepper mild mottle virus* on peppers and the disinfection of cutting tools in Taiwan. J. Taiwan Agric. Res. 67(4):347–354.

Pepper samples showed virus-like symptoms were collected from Kaohsiung and Pintung areas for virus detection by indirect enzyme-linked immunosorbent assay (indirect-ELISA) using antisera specific to nine viruses- *Capsicum chlorosis virus* (CaCV), *Chilli veinal mottle virus* (ChiVMV), *Cucumber mosaic virus* (CMV), *Pepper mild mottle virus* (PMMoV), *Pepper veinal mottle virus* (PVMV), *Potato virus Y* (PVY), *Tobacco mosaic virus* (TMV), *Tomato mosaic virus* (ToMV), and *Tomato spotted wilt virus* (TSWV). The results showed that ChiVMV was the most prevalent, followed by PVMV. Most samples detected were mix-infected with ChiVMV and PMMoV. Complex infections were likely to cause severe malformation of leaves and fruits, resulting in dwarfing and growth stagnation. In most cases, the symptoms caused by infections of more than two different viruses were more serious than that infected by a single virus. PMMoV could transmit by seed. Seed disinfection was a critical process in preventing virus spread during propagation and production. To directly address the concern, we estimate the efficacy of different disinfectants for treating virus-contaminated seeds. PMMoV can be transmitted through seeds. Direct seed-grinding and grown-out seedlings could be used to measure the PMMoV infection rate of seeds. PMMoV could be detected in 3 of 20 lots of pepper seeds in the grinding sap by ELISA. The PMMoV infection was measured and the virus infection rates were 13%, 18%, and 17%, respectively. The artificially PMMoV- contaminated seeds were placed in a fine mesh bag (100 bags) and soaked in 12.5%, 15%, and 20% trisodium phosphate solution for 10 min, 20 min, and 30 min, respectively, and then rinsed with running water for about 60 min. 1% sodium hypochlorite was used to remove the bacteria on the surface of the seed and put it on wet filter paper to make it germinate. The virus infection rates of the seedlings were investigated, in which the solution was treated with 12.5% trisodium phosphate solution for 30 min (the virus infection rate was 5%). Treatment with 15% trisodium phosphate solution for 30 min (virus infection rate 3%) and 20% trisodium phosphate solution for 20 min (virus infection rate 5%) had the best ability to remove PMMoV. In terms of equipment disinfection tests, the detoxification effect of 5% sodium hypochlorite and 0.5% sodium hypochlorite in three tests can reach 95% or more, followed by 20% skim milk powder.

Key words: *Pepper mild mottle virus*, Disinfection of cutting tools, Seed disinfection.

Received: October 20, 2017; Accepted: May 14, 2018.
[*] Corresponding author, e-mail: tsly@fthes-tari.gov.tw
[1] Assistant Research Fellow, Department of Plant Protection, Fengshan Tropical Horticultural Experiment Branch, Taiwan Agriculture Research Institute, Kaohsiung, Taiwan, ROC.

台灣農業研究 (J. Taiwan Agric. Res.) 67(4):355–364 (2018)
DOI:10.6156/JTAR.201812_67(4).0004

研究報告

香菇柄水萃物改善糖尿病降低阿茲海默氏症罹病風險初探

楊淑惠 [1,*] 楊惠婷 [2] 蔣淑琦 [3]

摘要

楊淑惠、楊惠婷、蔣淑琦。2018。香菇柄水萃物改善糖尿病降低阿茲海默氏症罹病風險初探。台灣農業研究 67(4):355–364。

阿茲海默氏症 (Alzheimer's disease; AD) 為慢性神經性退化疾病，本研究以糖尿病 (diabetes mellitus; DM) 誘發 AD 之動物模式，探討攝食香菇柄水萃物對 AD 危險因子的改善功效。研究顯示，大鼠攝食高脂飲食和注射低劑量 Streptozotocin，確實造成類似糖尿病，多食、多喝及體重減輕的病徵，也引發 AD 危險因子數量的增加。DM 大鼠攝食高劑量香菇蕈柄萃取物，可以有效改善血液生化值，增加胰島素分泌及腦部胰島素接受器的含量 ($P < 0.05$)，而顯著降低腦部 AD 危險因子類澱粉前驅蛋白 (amyloid precursor proteins; APP)、β-類澱粉蛋白-42 (β-amyloid-42; Aβ42)、β-分泌酶 (β-site amyloid precursor protein cleaving enzyme 1; BACE1)、糖化終產物 (advanced glycation endproducts; AGEs)、腫瘤壞死因子-α (tumor necrosis factor-α; TNF-α) 含量 ($P < 0.05$)。攝食香菇柄水萃物 DM 大鼠，進入十字迷宮開放手臂區和中心區的時間顯著增加 ($P < 0.05$)。由以上結果顯示，糖尿病動物攝食香菇柄水萃物可以改善糖尿病的生化代謝，進而降低 AD 罹病機率，或延緩後續病程發生。

關鍵詞：香菇柄水萃物、糖尿病、阿茲海默氏症、胰島素、舉臂式十字迷宮。

前言

阿茲海默氏症 (Alzheimer's disease; AD) 為慢性神經性退化疾病，患者臨床病理特徵為腦部神經纖維糾結 (neurofibrillary tangles; NFTs) 和大腦皮質及海馬迴會有大量的 β-類澱粉蛋白 (β-amyloid; Aβ) 沉澱而形成斑塊 (senile plaques)，進而導致腦神經細胞營養不足，造成突觸功能喪失、神經元死亡；腦部顳葉內側漸漸萎縮 (Gibson *et al*. 2003; Selkoe *et al*. 2004)。正子放射斷層攝影，發現 AD 患者腦部皮層的葡萄糖代謝速率較健康人緩慢，顯示出腦部葡萄糖攝取與 AD 病程有相關性 (Minoshima *et al*. 1997)。葡萄糖為大腦和神經細胞唯一的能量來源，神經細胞攝入葡萄糖需仰賴細胞膜上的胰島素接受器 (insulin receptor; ISR)，試驗動物處於醣類代謝異常，會損傷記憶和突觸的可塑性 (Tun *et al*. 1990; Stranahan *et al*. 2008)，在 AD 患者發現，腦部 Aβ 聚物可直接抑制 insulin/Akt 路徑，並減少樹突突觸的 ISR 的結合能力，對神經元造成胰島素阻抗 (De Felice *et al*. 2009)。糖尿病 (diabetes mellitus; DM) 病程中引發的氧化壓力和長期高血糖、胰島素分泌不足，會使 DM 患罹患 AD 的風險增加 2–3 倍 (Petot *et al*. 2003; Zhao & Townsend 2009)。AD 患者中有 80% 的人患有糖尿病，當體內長期處於高血糖，容易使醛基和胺基產生非酵素性褐變反應 (Maillard reaction) 而形成糖化終產物 (advanced glycation endproducts; AGEs)，堆積在血漿和腦部 (Rodriguez-Mañas *et al*. 2003)。以腹腔注射 Streptozotocin (STZ) 大鼠來誘發高血糖和胰島素異常，發

投稿日期：2018 年 3 月 21 日；接受日期：2018 年 5 月 26 日。
[*] 通訊作者：debbie@fthes-tari.gov.tw
[1] 農委會農業試驗所鳳山熱帶園藝試驗分所經營利用系副研究員兼主任。台灣 高雄市。
[2] 中國醫藥大學營養系副教授。台灣 台中市。
[3] 中國醫藥大學營養系研究生。台灣 台中市。

現動物體內 AGEs 和糖化終產物接受器 (receptor for advanced glycation endproducts; RAGE) 含量顯著增加，使氧化壓力提高；膜上類澱粉前驅蛋白 (amyloid precursor proteins; APP) 量增加，β-分泌酶 (β-site amyloid precursor protein cleaving enzyme 1; BACE1) 表現量增高，使得體內蓄積大量 Aβ (Ko *et al*. 2010; Cai *et al*. 2011)。焦慮緊張、易怒、注意力不集中、不安定，也常是 AD 病患在疾病中期出現的精神狀態，舉臂式十字迷宮 (elevated plus maze tests; EPM) 測試，是瞭解焦慮狀態常用的動物實驗模式 (Itoh *et al*. 1990; Carvalho *et al*. 2013)。本試驗以 DM 誘發 AD 之動物模式，探討攝食香菇蕈柄萃取物對 AD 指標因子，胰島素、血糖變化、AGEs、APP、β-類澱粉蛋白-42 (β-amyloid-42; Aβ42)、BACE1 等的改善效果，期待能對延緩 AD 病程有助益。

材料與方法

試驗動物

試驗動物為六週齡雄性 Wistar 大鼠共 60 隻 (購自樂斯科生物科技股份有限公司)，飼養於環境溫度 23℃ ± 1℃，濕度 40–60%，12 h 光暗循環的不銹鋼籠，經 1 wk 適應期後進行試驗。試驗動物分為兩大組：正常組和糖尿病誘發 AD 的 DM 組。正常組食用 Laboratory rodent diet 5001 (Purina Co., MO, USA)。DM 組餵食高脂飲食 (表 1) 及腹腔注射 40 mg kg $B.W.^{-1}$ 劑量 Streptozotocin (STZ) 誘發糖尿病，誘導 AD 發生。餵食香菇蕈柄萃取物為 DM 試驗組，口服低 (DM + L)、中 (DM + M)、高 (DM + H) 劑量香菇蕈柄萃取物 (劑量 0.16、0.48、0.96 g kg $B.W.^{-1}$)，試驗期 6 wk。試驗期間記錄大鼠攝食量、飲水量、體重及血糖值、胰島素變化，犧牲前禁食 12 h，犧牲後取大動脈血液及腦部組織，進行後續分析。

香菇柄萃取 (Lentinus edodes stipe extracts; LedoStE)

取適量乾燥香菇蕈柄 (購於台中市新社區阿亮香菇園)，加樣品量 5 倍水，冷藏復水 8 h，以高壓方式 (121℃) 進行 30 min 萃取，收集萃取液，冷凍乾燥後冷藏備用。

舉臂式十字迷宮 (Elevated plus-maze; EPM)

犧牲前對大鼠施行 EPM 試驗，十字迷宮為壓克力製成，試驗器材離地 50 cm，具有兩支開放手臂 (open arms；走道旁無壓克力壁) 和兩支封閉手臂 (close arms；走道旁有壓克力壁 35.5 cm)，兩兩手臂交會出一個中心空間 12 cm，其空間長 59 cm、寬 9 cm。試驗開始，將大鼠放置在離中心位置 15 cm 的開放手臂上，利用高空攝影機記錄老鼠進入中心位置、開放手臂與封閉手臂的次數和時間，整個試驗過程 5 min，將影像傳輸至電腦，利用動物行為觀察軟體 (Ethovision®XT Version 8.0, Noldus, Wageningen, The Netherlands) 進行分析。

血液分析

空腹血糖 (fasting blood glucose)：以乙醚迷昏採集尾巴血液，利用 EasiCheck 血糖試紙及血糖機測得血糖值。

血漿胰島素 (insulin)：使用 Rat Insulin kit ELISA (cat#10-1250-01, Mercodia AB, Uppsala, Sweden)，加入含有胰島素之血漿，胰島素與抗體結合形成抗體-抗原-酶標抗體複合物，洗滌後加 3,3’,5,5’-Tetramethylbenzidine (TMB) 顯色。TMB 在 Horseradish peroxidase (HRP) 的催化下轉化成藍色，並在酸的作用下轉化成黃色。ELISA reader 於 OD_{450nm} 測定吸光度，以

表 1. Laboratory rodent diet 5001 與高脂飲食之成分比例。
Table1. Composition of laboratory rodent diet 5001 and high fat diet.

Group	Laboratory rodent diet 5001	High fat diet
Carbohydrate (%)	58	52
Fat (%)	13	30
Protein (%)	29	18

標準曲線計算胰島素濃度。

血漿麩胺酸苯醋酸轉氨酶 (glutamate oxaloacetate transaminase; GOT)、血漿麩胺酸丙酮酸轉氨酶 (glutamate pyruvate transaminase; GPT)：採用 RANDOX Laboratories (Antrum, Northern Ireland) 公司之商業試劑組進行分析，以自動分析儀檢測 GOT 及 GPT。

總膽固醇 (Total cholesterol; TC)：使用商業試劑組 (cat#CH7201, RANDOX Laboratories, Antrum, Northern Ireland) 分析，以分光光度計 OD_{500nm} (Hitachi, Tokyo, Japan) 測量吸光值，換算 TC 濃度。三酸甘油酯 (Triglyceride; TG)，使用商業試劑組 (cat#TR213, RANDOX Laboratories, Antrum, Northern Ireland) 分析，以分光光度計 OD_{550nm} 測量吸光值，換算 TG 濃度。

腦部組織分析

APP、Aβ42、AGEs 及 ISR 測定，使用 Enzyme-lined Immunosorbent assay kit 分別為 USCNK, cat#E91020Ra、cat#E90946Ra、cat#E91353Ge 及 cat#E0895Ra，於 96 孔盤接上生物素標記的多細胞繁殖特定抗體。將含有特定抗體之試樣、標準品與各別之抗體結合，加入 HRP 標記的抗生物素蛋白後，加入 TMB 顯色以 ELISA reader 於 OD_{450nm} 測定吸光度，以各別之標準曲線計算其含量。

脂質過氧化 (丙二醛 malonaldehyde; MDA) 分析

取 100 μL 試樣混合 4 mL 0.2% H_2SO_4、0.5 mL 10% Phosphotungstic acid 和 1 mL 0.67% Thiobarbituric acid (溶於 H_2O : acetic acid = 1 : 1)，95℃水浴加熱。降溫後加入 Butanol 進行萃取，以 2,860× *g* 轉速離心取出 200 μL 上層液於螢光光度計 Ex515 nm/Em555 nm 測脂質過氧化值。

腫瘤壞死因子-α (Tumor necrosis factor-α; TNF-α)

使用 Enzyme-lined Immunosorbent assay kit (cat#ET2010-1, ASSAYPRO, St Charles, MO, USA)，以 ELISA reader 於 OD_{450nm} 測定吸光度，以標準曲線計算 TNF-α 含量。

BACE1 之西方墨點法

試樣進行蛋白質定量，調整蛋白質濃度到 8 μg μL^{-1}，取 10 μL 樣品並加入等量放射免疫沉澱法緩衝液 (radioimmunoprecipitation assay buffer; RIPA buffer)，乾熱器加熱。將樣品液轉移到 10% Acrylamide gel 電泳槽進行分離。完成後，切除 Stacking gel，取下 Separating gel 並浸泡在轉印緩衝液 (transfer buffer)；裁取相同大小的轉印模 (polyvinylidene difluoride membrane; PVDF) 泡於 99% Ethanol，依序放在三明治夾式塑膠板固定，放入濕式轉印槽，以 300 mA 進行轉印。完成轉印，取出 PVDF 膜以緩衝液沖洗；接續浸泡於 Blocking solution，沖洗後於 4℃浸泡初級抗體至隔夜；沖洗後以 2 級抗體室溫壓 1 h，沖洗後加入 ECL solution (Amersham Pharmacia Biotech, Amersham, UK) 呈色，以冷光螢光數位照相系統 (LAS-4000 mini, Fujifilm, Tokyo, Japan) 拍照。

α-生育醇 (α-tocopherol)

取 1 mL 腦組織均質液，先加入 600 μL 含 50 μg mL^{-1} α-tocopheryl acetate (cat#58957, Sigma-Aldrich, St. Louis, MO, USA) 的甲醇溶液震盪萃取 1 min，再加入 2 mL Hexane 震盪萃取 1 min，於 4℃、2,860× *g* 離心 30 min。收集上層液至另一微量離心管，抽乾溶劑後用 350 μL 甲醇回溶，並以真空過濾裝置用 0.45 μm 濾膜過濾，將過濾液收集於微量離心管中，抽取 80 μL 以高效液相層析儀 (high performance liquid chromatography; HPLC) 分析 α-生育醇含量。以標準曲線所得到的斜率，換算 α-生育醇濃度 (μg g^{-1} 腦重量)。

統計分析

試驗數據以平均值標準偏差 (mean ± SEM) 表示，採用 SPSS 12.0 軟體分析。試驗結果以單因子變異分析 (one-way analysis of variance; ANOVA) 進行分析，若達到顯著差異時，再以 Duncan’s multiple range test 進行組間差異確認，$P < 0.05$ 代表有顯著差異。

結果

試驗期間 DM 大鼠攝取香菇柄水萃物於對攝食量、飲水量、體重及血糖的影響

大鼠攝取高脂飲食及注射低劑量 STZ 確實誘發糖尿病 (DM) 發生，如表 2 所示。DM 組大鼠的攝食量顯著高於正常組 2–3 倍；飲水量顯著高於正常組 4–5 倍；但體重卻不增反減，成長率呈現正負值，惟攝取高劑量香菇柄水萃物組 (DM + H) 體重正成長 0.54%。試驗期間正常組的血糖值 97.9 ± 3.4 mg dL^{-1}–119.4 ± 1.5 mg dL^{-1}，DM 組的血糖值 394.5 ± 13.6 mg dL^{-1}–406.1 ± 10.9 mg dL^{-1}，顯著性高於正常組 (表 3)。DM 試驗組攝取 LedoStE 第 2 週，DM + M 及 DM + H 組的血糖值均下降至低於 DM 組；繼續攝食至第 3 週，血糖值 349.8 ± 15.8 mg dL^{-1}–364.2 ± 25.6 mg dL^{-1} 均低於 DM 組而達顯著差異 (P < 0.05)。如表 4 所示，試驗期間正常組胰島素量 1.37 ± 0.07 μg L^{-1}–2.63 ± 0.28 μg L^{-1}，DM 組胰島素量 0.18 ± 0.06 μg L^{-1}–0.65 ± 0.06 μg L^{-1}，顯著低於正常組。DM 試驗組攝食 LedoStE 第 3 週，胰島素量 1.31 ± 0.05 μg L^{-1}–1.66 ± 0.26 μg L^{-1}，均高於 DM 組而達顯著差異 (P < 0.05)；至第 6 週 DM + H 組胰島素量 1.71 ± 0.58 μg L^{-1}，與正常組無顯著差異 (P > 0.05)，顯示攝食 LedoStE 對糖尿病動物有正向影響。

DM 大鼠攝取香菇柄水萃取物對 EPM 表現的影響

EPM 為 AD 藥物篩選常用的動物評估模式 (Belzung & Griebel 2001; Cheng *et al*. 2007; Harris *et al*. 2010; Webster *et al*. 2013)。EPM 總觀察時間為 301 s，DM 組在封閉手臂區的時間 193.43 ± 75.82 s，占總觀察時間的 64.24 ± 25.18%，高於正常組 17.49 ± 12.05%；而在開放

表 2. DM 大鼠攝取香菇柄水萃物的攝食量、飲水量、體重的影響。

Table 2. Effects of eating stipe extracts of *Lentinus edodes* on food intake, water intake and body weight of DM rat.

Item	NC[z]	DM	DM + L	DM + M	DM + H
Initial body Weight (g)	443.60 ± 11.40 a[y]	426.90 ± 11.60 a	411.00 ± 11.60 a	398.00 ± 5.80 a	405.90 ± 17.70 a
Final body weight (g)	468.90 ± 12.00 a	405.00 ± 17.30 b	406.40 ± 10.50 b	396.80 ± 10.70 b	414.00 ± 17.40 b
Growth rate (%)	1.00 ± 1.00	-1.13 ± 0.49	-0.22 ± 1.24	-0.07 ± 1.43	0.54 ± 1.49
Food intake (g d^{-1})	24.90 ± 3.10 b	53.71 ± 4.43 a	56.42 ± 7.71 a	60.60 ± 5.35 a	52.14 ± 5.71 a
Water intake (mL d^{-1})	49.06 ± 10.45 b	251.20 ± 51.13 a	254.10 ± 51.50 a	243.00 ± 87.80 a	232.20 ± 33.50 a

[z] NC: normal control rat; DM: diabetes mellitus rat; DM + L: DM rate with 0.16 g kg^{-1} B.W. LedoStE; DM + M: DM rate with 0.48 g kg^{-1} B.W. LedoStE; DM + H: DM rate with 0.96 g kg^{-1} B.W. LedoStE.

[y] Values are presented as mean ± SEM. Values with different superscript letters are significantly different (P < 0.05).

表 3. DM 大鼠攝取香菇柄水萃物對血糖值的影響。

Table 3. Effects of eating stipe extracts of *Lentinus edodes* on blood glucose of DM rat.

Item	NC[z]	DM	DM + L	DM + M	DM + H
Initial experiment	119.4 ± 1.5 c[y]	400.8 ± 25.4 a	399.2 ± 18.1 a	406.1 ± 10.9 a	394.5 ± 13.6 a
Week 1	97.9 ± 3.4 c	392.3 ± 22.2 a	346.9 ± 17.1 a	378.9 ± 24.0 a	346.3 ± 15.0 a
Week 2	102.9 ± 2.2 c	445.8 ± 25.4 a	385.3 ± 19.6 ab	339.7 ± 30.2 b	343.9 ± 20.7 b
Week 3	104.6 ± 4.3 c	428.4 ± 8.3 a	364.2 ± 25.6 b	361.8 ± 25.9 b	349.8 ± 15.8 b
Week 4	99.6 ± 4.6 c	418.5 ±7.5 a	347.5 ± 25.5 b	331.1 ± 28.4 b	337.4 ± 22.6 b
Week 5	109.1 ± 3.9 c	435.1 ± 10.9 a	343.6 ± 24.2 b	341.1 ± 14.9 b	343.4 ± 17.8 b
Week 6	104.4 ± 3.0 c	400.0 ± 7.5 a	317.0 ± 18.1 b	318.4 ± 23.2 b	304.8 ± 16.9 b

[z] NC: normal control rat; DM: diabetes mellitus rat; DM + L: DM rate with 0.16 g kg^{-1} B.W. LedoStE; DM + M: DM rate with 0.48 g kg^{-1} B.W. LedoStE; DM + H: DM rate with 0.96 g kg^{-1} B.W. LedoStE.

[y] Values are presented as mean ± SEM. Values with different superscript letters are significantly different (P < 0.05).

表 4. DM 大鼠攝取香菇柄水萃物的胰島素含量的影響。

Table 4. Effects of eating stipe extracts of *Lentinus edodes* on insulin of DM rat.

Item	NC[z]	DM	DM + L	DM + M	DM + H
Initial experiment	1.37 ± 0.07 a[y]	0.47 ± 0.01 b	0.50 ± 0.01 b	0.49 ± 0.02 b	0.52 ± 0.04 b
Week 1	2.19 ± 0.40 a	0.65 ± 0.02 b	0.75 ± 0.10 b	0.87 ± 0.21 b	0.82 ± 0.10 b
Week 2	1.60 ± 0.47 a	0.41 ± 0.05 b	0.46 ± 0.07 b	0.71 ± 0.20 b	0.82 ± 0.16 ab
Week 3	2.36 ± 0.23 a	0.18 ± 0.06 c	1.45 ± 0.11 b	1.31 ± 0.05 b	1.66 ± 0.26 b
Week 4	2.44 ± 0.25 a	0.42 ± 0.17 c	1.43 ± 0.25 b	1.45 ± 0.18 b	1.84 ± 0.45 a
Week 5	2.24 ± 0.35 a	0.47 ± 0.06 c	1.39 ± 0.17 b	1.60 ± 0.13 b	1.66 ± 0.35 b
Week 6	2.63 ± 0.28 a	0.57 ± 0.02 c	1.03 ± 0.04 b	1.44 ± 0.49 b	1.71 ± 0.58 a

[z] NC: normal control rat; DM: diabetes mellitus rat; DM + L: DM rate with 0.16 g kg^{-1} B.W. LedoStE; DM + M DM rate with 0.48 g kg^{-1} B.W. LedoStE; DM + H: DM rate with 0.96 g kg^{-1} B.W. LedoStE.
[y] Values are presented as mean ± SEM. Values with different letters are significantly different ($P < 0.05$).

手臂區及中心區的時間分別為 21.79 ± 20.76% 及 13.96 ± 8.37%，則低於正常組 54.47 ± 12.06% 及 28.04 ± 8.31%，DM 組 EPM 表現相對焦慮。DM 試驗組攝食不同劑量 LedoStE 6 wk，在開放手臂區的時間占總觀察時間的 44.19 ± 8.88%–50.88 ± 21.3% 及在中心區的時間 27.82 ± 16.39%–34.21 ± 24.76%，均顯著高於 DM 組 ($P < 0.05$)，而與正常組無顯著差異 ($P > 0.05$)。如表 5 所示，顯示 DM 組的活動力獲得改善。

DM 大鼠攝取香菇柄水萃物對血液値的影響

DM 大鼠血液生化值，如表 6 所示，Aspartate transaminase (AST)、Alanine transaminase (ALT) 值高於正常組 2–3 倍；TC、TG 值高於正常組 1.5–4.5 倍；AGEs 量高於正常組 5 倍，數值顯示 DM 大鼠體內發炎、脂質及過氧化物質含量過高，AGEs 大量累積，及 ISR 含量不足的狀態。DM 大鼠攝食不同劑量 LedoStE 6 wk，血液生化值均顯著改善，DM + H 組 TC 值 59.00 ± 9.17 mg dL^{-1}，MDA 值 9.11 ± 0.61 μM 及 ISR 值 17.32 ± 2.03 ng dL^{-1}，則與正常組則無顯著差異 ($P > 0.05$)，顯示糖尿病動物生化代謝獲得改善。

DM 大鼠攝取香菇柄水萃物對腦部誘發 AD 危險因子的影響

大鼠攝取高脂飲食及注射低劑量 STZ 確實誘發 DM，並誘導 AD 危險因子顯著增加。如表 7 所

表 5. DM 大鼠攝取香菇柄水萃物的十字迷宮表現的影響。

Table 5. Effects of eating stipe extracts of *Lentinus edodes* on elevated plus-maze performance of DM rat.

Item	NC[z]	DM	DM + L	DM + M	DM + H
Close arms					
Time (s)[y]	52.67 ± 36.28 b[x]	193.43 ± 75.82 a	64.12 ± 74.47 b	72.01 ± 29.68 b	54.80 ± 43.26 b
Time (%)	17.49 ± 12.05 b	64.24 ± 25.18 a	21.30 ± 24.73 b	23.92 ± 9.86 b	18.20 ± 14.37 b
Open arms					
Time (s)	164.00 ± 36.30 a	65.61 ± 62.51 b	153.21 ± 64.26 a	133.06 ± 26.75 a	143.27 ± 63.79 a
Time (%)	54.47 ± 12.06 a	21.79 ± 20.76 b	50.88 ± 21.34 a	44.19 ± 8.88 a	47.58 ± 21.19 a
Center					
Time (s)	84.42 ± 25.02 a	42.03 ± 25.19 b	83.77 ± 49.36 a	96.03 ± 27.07 a	103.01 ± 74.56 a
Time (%)	28.04 ± 8.31 a	13.96 ± 8.37 b	27.82 ± 16.39 a	31.89 ± 8.99 a	34.21 ± 24.76 a

[z] NC: normal control rat; DM: diabetes mellitus rat; DM + L: DM rate with 0.16 g kg^{-1} B.W. LedoStE; DM + M: DM rate with 0.48 g kg^{-1} B.W. LedoStE; DM + H: DM rate with 0.96 g kg^{-1} B.W. LedoStE.
[y] Total time (s) = 301.1
[x] Values are presented as mean ± SEM. Values with different letters are significantly different ($P < 0.05$).

表 6. DM 大鼠攝取香菇柄水萃物對血液生化值的影響。

Table 6. Effects of eating stipe extracts of *Lentinus edodes* on blood parameter of DM rat.

Item	NC[z]	DM	DM + L	DM + M	DM + H
AST (U L^{-1})	65.75 ± 5.37 c[y]	157.30 ± 24.85 a	114.90 ± 11.50 b	101.30 ± 10.50 b	104.20 ± 7.80 b
ALT (U L^{-1})	42.13 ± 2.57 c	139.60 ± 20.90 a	110.00 ± 12.90 a	113.40 ± 10.30 a	92.75 ± 9.87 b
TC (mg dL^{-1})	60.00 ± 4.57 c	94.13 ± 7.46 a	87.30 ± 5.52 a	71.00 ± 5.04 b	59.00 ± 9.17 c
TG (mg dL^{-1})	51.00 ± 4.08 c	227.00 ± 29.40 a	131.90 ± 15.00 b	136.90 ± 17.90 b	120.20 ± 27.90 b
MDA (μM)	10.06 ± 0.37 b	13.15 ± 0.64 a	10.97 ± 0.49 b	10.26 ± 0.77 b	9.11 ± 0.61 b
AGEs (ng mL^{-1})	20.48 ± 2.68 b	98.29 ± 29.56 a	51.71 ± 10.24 b	47.87 ± 8.58 b	41.40 ± 6.46 b
ISR (ng dL^{-1})	15.37 ± 1.25 a	8.22 ± 1.64 c	15.74 ± 1.51 b	16.49 ± 2.90 a	17.32 ± 2.03 a

[z] NC: normal control rat; DM: diabetes mellitus rat; DM + L: DM rate with 0.16 g kg^{-1} B.W. LedoStE; DM + M: DM rate with 0.48 g kg^{-1} B.W. LedoStE; DM + H: DM rate with 0.96 g kg^{-1} B.W. LedoStE; AST: Aspartate transaminase; ALT: Alanine transaminase; TC: total cholesterol; TG: Triglyceride; MDA: malonaldehyde; AGEs: advanced glycation endproducts; ISR: insulin receptor.

[y] Values are presented as mean ± SEM. Values with different letters are significantly different ($P < 0.05$).

表 7. DM 大鼠攝取香菇柄水萃物對腦部 AD 危險因子的影響。

Table 7. Effects of eating stipe extracts of *Lentinus edodes* on AD risk factor in brain of DM rat.

Item	NC[z]	DM	DM + L	DM + M	DM + H
APP (ng mg^{-1} protein)	2.79 ± 0.13 b[y]	3.68 ± 0.15 a	3.30 ± 0.16 a	3.33 ± 0.12 a	2.67 ± 0.09 b
Aβ42 (pg mg^{-1} protein)	4.18 ± 0.88 c	12.61 ± 1.33 a	8.40 ± 0.70 b	8.87 ± 0.71 b	7.29 ± 1.16 b
AGEs (ng mg^{-1} protein)	98.70 ± 16.40 b	281.00 ± 61.70 a	127.70 ± 26.00 b	113.20 ± 12.60 b	110.80 ± 26.10 b
TNF-α (pg mg^{-1})	5.95 ± 0.39 c	9.20 ± 0.79 a	7.62 ± 0.48 b	7.04 ± 0.48 bc	6.82 ± 0.27 bc
MDA (μg g^{-1})	241.70 ± 6.50 c	322.70 ± 13.80 a	261.60 ± 7.60 b	270.30 ± 6.40 b	270.40 ± 7.90 b
α-tocopherol (μg mg^{-1})	0.70 ± 0.06 a	0.28 ± 0.03 b	0.55 ± 0.04 a	0.53 ± 0.06 a	0.61 ± 0.06 a
ISR (ng mg^{-1} protein)	1.26 ± 0.29 a	0.54 ± 0.17 c	0.97 ± 0.15 b	1.11 ± 0.09 a	1.15 ± 0.19 a

[z] NC: normal control rat; DM: diabetes mellitus rat; DM + L: DM rate with 0.16 g kg^{-1} B.W. LedoStE; DM + M: DM rate with 0.48 g kg^{-1} B.W. LedoStE; DM + H: DM rate with 0.96 g kg^{-1} B.W. LedoStE; APP: amyloid precursor proteins; Aβ42: β-amyloid-42; AGEs: advanced glycation endproducts; TNF-α: tumor necrosis factor-α; MDA: malonaldehyde; ISR: insulin receptor.

[y] Values are presented as mean ± SEM. Values with different letters are significantly different ($P < 0.05$).

示，DM 組 APP 含量 3.68 ± 0.15 ng mg $protein^{-1}$，顯著高於正常組 2.79 ± 0.13 ng mg $protein^{-1}$，DM + H 組 APP 量 2.67 ± 0.09 ng mg $protein^{-1}$ 與正常組無顯著差異 ($P > 0.05$)。DM 組 Aβ42 含量 12.61 ± 1.33 pg mg $protein^{-1}$，高於正常組 4.18 ± 0.88 pg mg $protein^{-1}$ 近 3 倍，攝食 LedoStE 各試驗組 Aβ42 量 7.29 ± 1.16 pg mg $protein^{-1}$–8.87 ± 0.71 pg mg $protein^{-1}$，均低於 DM 組，惟不同攝食劑量間差異不顯著 ($P > 0.05$)。DM 組 AGEs 量 281.0 ± 61.7 ng mg $protein^{-1}$ 顯著高於正常組 98.70 ± 16.4 ng mg $protein^{-1}$ 近 3.5 倍，攝食 LedoStE 各試驗組 AGEs 量 110.8 ± 26.1 ng mg $protein^{-1}$–127.7 ± 26.0 ng mg $protein^{-1}$ 均顯著低於 DM 組 ($P < 0.05$)，而與正常組無顯著差異 ($P > 0.05$)，惟不同攝食劑量間差異不顯著。DM 組 TNF-α 量 9.20 ± 0.79 pg mg^{-1} 顯著高於正常組 5.95 ± 0.39 pg mg^{-1}，DM + M、DM + H 組腦部 TNF-α 量分別為 7.04 ± 0.48 pg mg^{-1} 及 6.82 ± 0.27 pg mg^{-1} 均顯著低於 DM 組 ($P < 0.05$)，與正常組則無顯著差異 ($P > 0.05$)。DM 組 BACE1 量 1.150 ± 0.030 顯著高於正常組 1.000 ± 0.001，如圖 1 所示。DM 試驗組攝食不同劑量 LedoStE 6 wk，腦部 BACE1 量 1.020 ± 0.020–1.054 ± 0.050 均低於 DM 組；DM + H 組則與正常組則無顯著差異 ($P > 0.05$)，顯示誘發 AD 危險因子顯著下降。

DM 大鼠腦部脂質過氧化 (MDA)

MDA 為脂質過氧化指標，正常組大鼠腦部 MDA 含量 241.7 ± 6.5 μg g^{-1}，顯著低於 DM 組 322.7 ± 13.8 μg g^{-1} (表 7)。攝食 LedoStE 各試驗

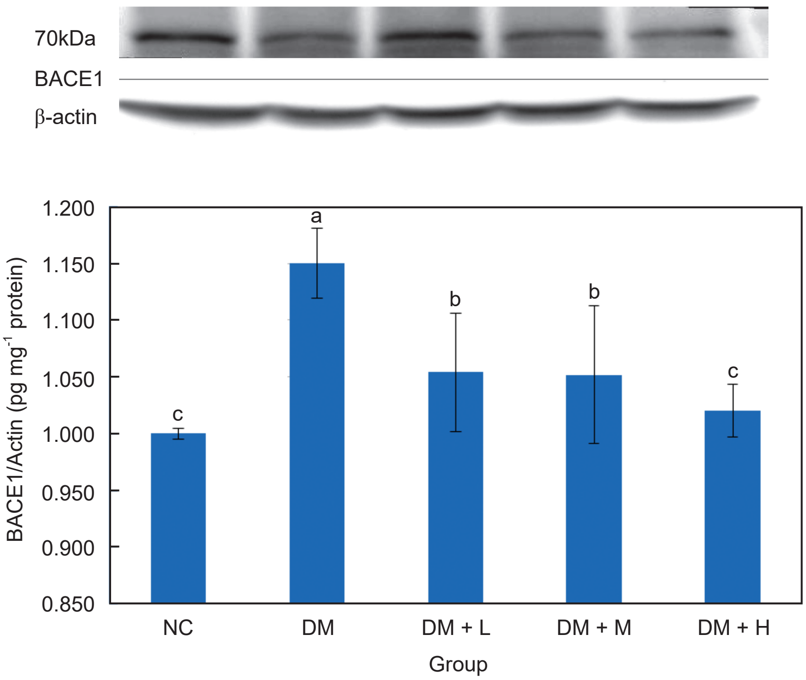

圖 1. DM 大鼠攝食菇柄水萃物對腦部 BACE1 因子的影響。

Fig. 1. Effects of eating stipe extracts of *Lentinus edodes* on β-site amyloid precursor protein cleaving enzyme 1 (BACE1) factor in brain of diabetes mellitus (DM) rat. NC: normal control rat; DM: diabetes mellitus rat; DM + L: DM rate with 0.16 g kg^{-1} B.W. LedoStE; DM + M: DM rate with 0.48 g kg^{-1} B.W. LedoStE; DM + H: DM rate with 0.96 g kg^{-1} B.W. LedoStE. Values are presented as mean ± SEM. Values with different superscript letters are significantly different ($P < 0.05$).

組 MDA 含量 261.6 ± 7.6 μg g^{-1}–270.4 ± 7.9 μg g^{-1} 均低於 DM 組，脂質過氧化情形下降，惟不同攝食劑量間差異不顯著 ($P > 0.05$)。

α-生育醇

α-生育醇含量為抗氧化正相指標，正常組大鼠腦部 α-生育醇含量 0.70 ± 0.06 μg mg^{-1}，顯著高於 DM 組 0.28 ± 0.03 μg mg^{-1} 近 3.5 倍 (表 7)。攝食 LedoStE 各試驗組 α-生育醇含量 0.53 ± 0.06 μg mg^{-1}–0.61 ± 0.06 μg mg^{-1} 均高於 DM 組，與正常組則無顯著差異 ($P > 0.05$)，顯示抗氧化力增加，惟不同攝食劑量間差異不顯著。

ISR

ISR 含量為正向指標，正常組大鼠腦部 ISR 含量 1.26 ± 0.29 ng mg $protein^{-1}$，顯著高於 DM 組 0.54 ± 0.17 ng mg $protein^{-1}$ 近 2 倍 (表 7)。攝食 LedoStE 各試驗組腦部 ISR 含量 0.97 ± 0.15 ng mg $protein^{-1}$–1.15 ± 0.19 ng mg $protein^{-1}$ 均高於 DM 組；DM + M、DM + H 組則與正常組則無顯著差異 ($P > 0.05$)，顯示 ISR 含量增加。

討論

大鼠攝取高脂飲食和注射低劑量 STZ，確實誘發造成類似糖尿病多食、多喝及體重減輕的病徵，也引發 AD 危險因子含量的增加。DM 大鼠攝食高劑量香菇蕈柄水萃物，可以有效改善脂質過高、發炎現象，並降低氧化傷害，改善血糖值及胰島素分泌；也顯著降低誘發 AD 危險因子 APP、Aβ42、BACE1、AGEs、TNF-α 及脂質過氧化質的含量。而增加 α-生育醇、ISR 的含量及大鼠的活動力。文獻顯示，攝食香菇多醣 (lentinan)，可顯著增加大鼠血清 Superoxide dismutase (SOD)、Glutathione peroxidase (GSH-Px) 並且降低 Interleukin-1β (IL-1β)、TNF-α 和減少潰瘍面積 (Yu *et al*. 2009)。香菇多酚物質，可提高整體抗氧化活性及降低化學

物質誘發的肝臟發炎現象 (Akamatsu *et al*. 2004; Choi *et al*. 2006)；香菇嘌呤 (eritadenine) 可調節 Δ6-Desaturase 活性，使體內脂質顯著降低至恆定，減少所攝食油脂轉成三酸甘油酯 (Shimada *et al*. 2002)；麥角硫因 (ergothioneine) 可以有效抑制 Nuclear factor-κB (NF-κB) 的活性，減少發炎現象 (Rahman *et al*. 2003)；報告顯示 AD 患者腦部的膽固醇含量及 β-secretase、γ-secretase 活性顯著高於正常組，N2a 細胞試驗顯示，高膽固醇含量與 β-secretase 活性及 Aβ 分泌增加有關。麥角固醇 (ergosterol) 用量在 20–80 μM 時，可稍微降低 β-secretase 活性；用量增加為 40 μM 時會強烈抑制 γ-secretas 活性 (Xiong *et al*. 2008)。

香菇柄水萃物成分析顯示含有許多生物活性成分，包括類黃酮 (flavonoid)、酚酸化合物 (phenolic acid)、香菇多醣 (lentinan)、香菇嘌呤、麥角硫因、三萜類 (triterpen)、肌醇 (inositol)、麥角固醇等，如表 8 所示，均可能有助於改善糖尿病動物的生化代謝病程，進而降低 AD 罹病機率，或延緩後續病程的發生。惟香菇柄水萃物的有效萃取指標成分尚有待確立；攝取水萃物對糖尿病動物的血液生化指標有改善，但截至實驗結束，對糖尿病動物多食、多喝及體重減輕病徵的改善不顯著；改善焦慮情緒是否也有助於記憶損傷的減緩等均有待進一步探討。

表 8. 香菇柄水萃物成分。
Table 8. The composition of *Lentinus edodes* stipe extracts.

Item	T-phenol equ. gallic	Flavonoid equ quercetin	Triterpene	Ergothioneine	Ergosterol	Eritadenine
mg g^{-1} ext	8.51 ± 0.00	3.29 ± 0.05	9.90 ± 0.17	1.14 ± 0.02	31.20 ± 0.13	5.97 ± 0.05

引用文獻

Akamatsu, S., A. Watanabe, M. Tamesada, R. Nakamura, S. Hayashi, D. Kodama, M. Kawase, and K. Yagi. 2004. Hepatoprotective effect of extracts from *Lentinus edodes* mycelia on dimethylnitrosamine-induced liver injury. Biol. Pharm. Bull. 27:1957–1960.

Belzung, C. and G. Griebel. 2001. Measuring normal and pathological anxiety-like behaviour in mice: A review. Behav. Brain Res. 125:141–149.

Cai, Z., Y. Zhao, S. Yao, and B. Zhao. 2011. Increases in β-amyloid protein in the hippocampus caused by diabetic metabolic disorder are blocked by minocycline through inhibition of NF-κB pathway activation. Pharmacol. Rep. 63:381–391.

Carvalho, C., N. Machado, P. C. Mota, S. C. Correia, S. Cardoso, R. X. Santos, M. S. Santos, C. R. Oliveira, and P. I. Moreira. 2013. Type 2 diabetic and Alzheimer's disease mice present similar behavioral, cognitive, and vascular anomalies. J. Alzheimers Dis. 35:623–635.

Cheng, I. H., K. Scearce-Levie, J. Legleiter, J. J. Palop, H. Gerstein, N. Bien-Ly, J. Puoliväli, S. Lesné, K. H. Ashe, P. J. Muchowski, and L. Mucke. 2007. Accelerating amyloid-β fibrillization reduces oligomer levels and functional deficits in Alzheimer disease mouse models. J. Biol. Chem. 282:23818–23828.

Choi, Y., S. M. Lee, J. Chun, H. B. Lee, and J. Lee. 2006. Influence of heat treatment on the antioxidant activities and polyphenolic compounds of Shiitake (*Lentinus edodes*) mushroom. Food Chem. 99:381–387.

De Felice, F. G., M. N. Vieira, T. R. Bomfim, H. Decker, P. T. Velasco, M. P. Lambert, K. L. Viola, W. Q. Zhao, S. T. Ferreira, and W. L. Klein. 2009. Protection of synapses against Alzheimer's-linked toxins: Insulin signaling prevents the pathogenic binding of Abeta oligomers. Proc. Natl. Acad. Sci. U. S. A. 106:1971–1976.

Gibson, W. W., G. P. Eckert, U. Igbavboa, and W. E. Müller. 2003. Amyloid beta-protein interactions with membranes and cholesterol: Causes or casualties of Alzheimer's disease. Biochim. Biophys. Acta. 1610:281–290.

Harris, J. A., N. Devidze, B. Halabisky, I. Lo, M. T. Thwin, G. Q. Yu, D. E. Bredesen, E. Masliah, and L. Mucke. 2010. Many neuronal and behavioral impairments in transgenic mouse models of Alzheimer's disease are independent of caspase cleavage of the amyloid precursor protein. J. Neurosci. 30:372–381.

Itoh, J., T. Nabeshima, and T. Kameyama. 1990. Utility of an elevated plus-maze for the evaluation of memory in mice: Effects of nootropics, scopolamine and electroconvulsive shock. Psychopharmacology (Berl.) 101:27–33.

Ko, S. Y., Y. P. Lin, Y. S. Lin, and S. S. Chang. 2010. Advanced glycation end products enhance amyloid precursor protein expression by inducing reactive oxygen species. Free Radic. Biol. Med. 49:474–480.

Minoshima, S., B. Giordani, S. Berent, K. A. Frey, N. L. Foster, and D. E. Kuhl. 1997. Metabolic reduction in the posterior cingulate cortex in very early Alzheimer's disease. Ann. Neurol. 42:85–94.

Petot, G. J., F. Traore, S. M. Debanne, A. J. Lerner, K. A. Smyth, and R. P. Friedland. 2003. Interactions of apolipoprotein E genotype and dietary fat intake of healthy older persons during mid-adult life. Metabolism 52:279–281.

Rahman, I., P. S. Gilmour, L. A. Jimenez, S. K. Biswas, F. Antonicelli, and O. I. Aruoma. 2003. Ergothioneine inhibits oxidative stress- and TNF-α-induced NF-κB activation and interleukin-8 release in alveolar epithelial cells. Biochem. Biophys. Res. Commun. 302:860–864.

Rodriguez-Mañas, L., J. Angulo, S. Vallejo, C. Peiró, A. Sánchez-Ferrer, E. Cercas, P. López-Dóriga, and C. F. Sánchez-Ferrer. 2003. Early and intermediate Amadori glycosylation adducts, oxidative stress, and endothelial dysfunction in the streptozotocin-induced diabetic rats vasculature. Diabetologia 46:556–566.

Selkoe, D. J., American College of Physicians, and American Physiological Society. 2004. Alzheimer disease: Mechanistic understanding predicts novel therapies. Ann. Intern. Med. 140:627–638.

Shimada, Y., T. Morita, and K. Sugiyama. 2002. Effects of dietary eritadenine on Δ6-desaturase activity and fatty acid profiles of several lipids in rats fed different fats. Biosci. Biotechnol. Biochem. 66:1605–1609.

Stranahan, A. M., T. V. Arumugam, R. G. Cutler, K. Lee, J. M. Egan, and M. P. Mattson. 2008. Diabetes impairs hippocampal function through glucocorticoid-mediated effects on new and mature neurons. Nat. Neurosci. 11:309–317.

Tun, P. A., D. M. Nathan, and L. C. Perlmuter. 1990. Cognitive and affective disorders in elderly diabetics. Clin. Geriatr. Med. 6:731–746.

Webster, S. J., A. D. Bachstetter, and L. J. Van Eldik. 2013. Comprehensive behavioral characterization of an APP/PS-1 double knock-in mouse model of Alzheimer's disease. Alzheimers Res. Ther. 5:28.

Xiong, H., D. Callaghan, A. Jones, D. G. Walker, L. F. Lue, T. G. Beach, L. I. Sue, J. Woulf, H. Xu, D. B. Stanimirovic, and W. Zhang. 2008. Cholesterol retention in Alzheimer's brain is responsible for high β- and γ-secretase activities and Aβ production. Neurobiol. Dis. 29:422–437.

Yu, Z. H., L. H. Yin, Y. Qian, and L. Yan. 2009. Effect of *Lentinus edodes* polysaccharide on oxidative stress, immunity activity and oral ulceration of rats stimulated by phenol. Carbohydr. Polym. 75:115–118.

Zhao, W. Q. and M. Townsend. 2009. Insulin resistance and amyloidogenesis as common molecular foundation for type 2 diabetes and Alzheimer's disease. Biochim. Biophys. Acta 1792:482–496.

Preliminary Study of *Lentinus edodes* Stipe Extracts on Reducing the Risk of Alzheimer's Disease in Diabetic Animal Model

Shu-Hui Yang[1,*], Hui-Ting Yang[2], and Shu-Chi Chiang[3]

Abstract

Yang, S. H., H. T. Yang, and S. C. Chiang. 2018. Preliminary study of *Lentinus edodes* stipe extracts on reducing the risk of Alzheimer's disease in diabetic animal model. J. Taiwan Agric. Res. 67(4):355–364.

Alzheimer's disease (AD) is a chronic neurodegenerative disease. In this study, animal model of diabetes induced AD was studied to investigate the effect of *Lentinus edodes* stipe extracts (LedoStE) on improving the risk factors of AD. Resultss showed that rats fed a high-fat diet and injected low-dose streptozotocin did cause diabetes-like symptoms-eating more, drinking more and weight loss, and also led to increase of AD risk factors. Diabetes mellitus (DM) rats fed with high-dose LedoStE can effectively improve the blood parameter as well as increase insulin secretion and insulin receptor content of brain ($P < 0.05$), while significantly decreased the levels of AD risk factors sash as amyloid precursor proteins (APP), β-amyloid-42 (Aβ42), β-site amyloid precursor protein cleaving enzyme 1 (BACE1), advanced glycation endproducts (AGEs), and tumor necrosis factor-α (TNF-α) ($P < 0.05$) in brain. It also significantly increased the time ($P < 0.05$) to enter the open and central areas of the elevated plus-maze in DM rats taking LedoStE. From the above results, it shows that diabetic animals intake LedoStE can improve the biochemical metabolism of diabetes, which may reduce the risk of Alzheimer's disease or delay the AD onset.

Key words: *Lentinus edodes* stipe extracts, Diabetes mellitus, Alzheimer's disease, Insulin, Elevated plus-maze.

Received: March 21, 2018; Accepted: May 26, 2018.

[*] Corresponding author, e-mail: debbie@fthes-tari.gov.tw
[1] Associate Research Fellow and Head, Department of Management and Utilization, Fengshan Tropical Horticultural Experimental Branch, Taiwan Agricultural Research Institute, Kaohsiung, Taiwan, ROC.
[2] Associate Professor, Department of Nutrition, China Medical University, Taichung, Taiwan, ROC.
[3] Graduate Student, Department of Nutrition, China Medical University, Taichung, Taiwan, ROC.

台灣農業研究 (J. Taiwan Agric. Res.) 67(4):365–376 (2018)
DOI:10.6156/JTAR.201812_67(4).0005 研究報告

評估氯化鐵清洗法對含鎘水稻田土壤鎘移除、土壤性質及稻作生長之影響

許健輝[1,*] 林毓雯[2] 鄭佳怡[3] 何雅婷[3] 陳柱中[1] 劉滄棽[2] 郭鴻裕[4]

摘要

許健輝、林毓雯、鄭佳怡、何雅婷、陳柱中、劉滄棽、郭鴻裕。2018。評估氯化鐵清洗法對含鎘水稻田土壤鎘移除、土壤性質及稻作生長之影響。台灣農業研究 67(4):365–376。

水稻田鎘汙染為嚴重的問題，因為攝食稻米為亞洲地區暴露鎘的主要途徑，所以降低水稻吸收土壤中的鎘對於減少人體暴露鎘的風險是相當重要的。土壤清洗法被認為是鎘移除效率高的土壤復育技術，然而目前較少研究評估現地氯化鐵清洗後的土壤鎘移除效率及作物生長狀況。因此，本研究目的為評估現地氯化鐵土壤清洗法對土壤鎘移除、土壤性質及稻作生長的影響。本研究試驗田位於台灣台中市后里地區，三塊含鎘水稻田 (試驗田 A、B、C) 土壤鎘濃度分別為 2.13、1.58 及 1.90 mg kg^{-1}，土壤清洗前後以網格方式採集土壤樣本，並分析其土壤鎘濃度及土壤性質。此外，在土壤清洗後之試驗田種植水稻，測定其生長指標及植株中的鎘濃度。試驗結果指出，氯化鐵清洗後三塊試驗田土壤平均鎘濃度分別降低 36.8、46.3 及 61.0%。相較於清洗前之土壤性質，清洗後土壤 pH 值、電導度 (electrical conductivity; EC) 及有效性磷有顯著變化，土壤總氮、總碳及陽離子交換容量 (cation exchange capacity; CEC) 則沒有顯著差異。稻作生長的結果指出，相較於對照組，氯化鐵清洗後水稻地上部高度、地上部生質量及稻穀產量未顯著下降，並且發現植體鎘濃度有下降的趨勢。總結本研究結果，現地氯化鐵清洗法可有效降低土壤的鎘濃度，並且對稻作的生長及稻穀產量影響不顯著。儘管此方法會些微影響土壤性質，但可透過石灰及肥料的供應回復地力。因此，本研究建議現地氯化鐵清洗法對於移除水稻田土壤鎘為可行的復育技術。

關鍵詞：鎘、土壤清洗、氯化鐵、水稻、現地試驗。

前言

鎘在環境中具有高度的移動性及生物有效性，故被認為容易透過植物傳輸至動物食物鏈的重金屬汙染物 (Hong *et al*. 2008)。過去研究指出，人體平均每天攝食的鎘含量約為 30 μg (Figueroa 2008)，一旦人體累積過量的鎘將對健康產生不利的影響，例如導致貧血、高血壓、癌症、骨質疏鬆、腎及肺功能衰竭等症狀 (Satarug *et al*. 2003; Godt *et al*. 2006)。農田土壤中鎘的來源包括天然及人為活動兩種途徑，天然的途徑為火山活動、土壤母質及含鎘礦物風化作用及土壤沖蝕等方式釋放到土壤環境中 (Adamu & Nganje 2010)。由於一般土壤中平均的鎘濃度低，所以經由天然途徑造成土壤鎘汙染的可能性較低。人為活動導致土壤鎘汙染的途徑包括採礦、煉鐵、電鍍及顏料等工業廢水排放至農業灌溉系統，以及施用品質不佳的汙泥、堆肥及磷肥 (Schoeters *et al*. 2006; Kuo *et al*. 2007; Loganathan *et al*. 2008)。近年來，由於稻米鎘超

投稿日期：2018 年 5 月 2 日；接受日期：2018 年 6 月 1 日。
[*] 通訊作者：chsyu@tari.gov.tw
[1] 農委會農業試驗所農業化學組助理研究員。台灣 台中市。
[2] 農委會農業試驗所農業化學組副研究員。台灣 台中市。
[3] 農委會農業試驗所農業化學組研究助理。台灣 台中市。
[4] 農委會農業試驗所農業化學組研究員兼組長。台灣 台中市。

標的問題層出不窮，特別對於以稻米為主食的台灣，如何降低土壤中鎘的有效性及水稻對鎘的吸收及累積，為現今急需解決的問題。

過去的研究指出，常用來降低土壤中鎘有效性及作物 (水稻) 吸收的農藝管理方法包括土壤穩定法、水分管理及種植低鎘吸收能力之品種及作物。土壤穩定 (化學固定) 法為添加化學固定劑，藉由提升土壤的酸鹼度及形成溶解度低的沉澱 [例如 $Cd(OH)_2$] 來降低鎘的生物有效性。一般常被使用的化學固定劑包括石灰資材、磷酸鹽肥料、鹼性生物汙泥及黏土礦物等，形成溶解度低的碳酸鎘 ($CdCO_3$)、磷酸鎘 [$Cd_3(PO_4)_2$] 或氫氧化鎘 [$Cd(OH)_2$] 沉澱 (Bolan *et al*. 2003a, 2003b; Basta & McGown 2004; Hong *et al*. 2010; Sun *et al*. 2015)，土壤穩定法具成本較低廉及對環境較友善的優點 (Basta & McGown 2004)。水分管理為降低水稻田鎘有效性的重要方法之一，因為在浸水 (厭氧) 的條件下，易形成溶解度低的硫化鎘 (CdS) 沉澱物，降低鎘的有效性；反之，在缺水 (好氧) 的條件下則是形成溶解度高的硫酸鎘化合物 ($CdSO_4$)。另外，在浸水條件下土壤 pH 趨向中性，也可因增加黏土礦物對鎘的吸附能力及形成溶解度低的氫氧化鎘沉澱等方式降低鎘的有效性 (Arao *et al*. 2009)。因此，長期湛水的管理方式被認為可有效降低水稻對鎘的吸收 (Arao *et al*. 2009; Hu *et al*. 2013)。過去研究也指出稉稻品種 'Japonica' 對鎘的吸收能力低於秈稻品種 'Indica'，可透過品種的選擇來降低鎘米的風險 (Kato *et al*. 2010; Bolan *et al*. 2013)。此外，Lin *et al*. (2015) 的研究也發現，國內不同蔬菜作物對於鎘的吸收能力有所差異，例如葉菜類的鎘吸收能力較豆菜類及果菜類高。因此，在鎘汙染潛勢農地，也可透過作物種類的選擇來降低食用部位鎘超標的風險。

近年來，國內陸續發現土壤鎘濃度低於環境保護署管制標準的農地 (食用作物農地鎘管制標準為 5 mg kg^{-1})，種出的作物食用部位鎘濃度卻高於衛生福利部的限量標準之問題。此問題可先透過前述農藝管理方法處理，一旦發現土壤汙染程度高且農藝方法效果不顯著時，降低土壤鎘濃度勢必為下一步解決問題的策略。一般來說，降低土壤鎘濃度的方法包括耕犁法、排客土法、植生萃取及現地土壤清洗法，上述方法中，現地土壤清洗法被認為具有較高的鎘移除效率，並且能在較短的時間內恢復耕種之復育方法 (Makino *et al*. 2008)。現地土壤清洗法被認為適合在水稻田施作，因為此類農地通常在耕犁層下有一層不透水層 (犁底層)，可將清洗溶液維持在土壤表層，不容易向下滲漏 (Makino *et al*. 2007; Makino *et al*. 2008)。土壤清洗的試劑種類包含金屬鉗合劑、中性鹽類及強酸。Ethylenediamine tetraacetic acid (EDTA) 為金屬鉗合劑，過去研究指出其對鎘的移除效果佳，但 EDTA 在環境中具有不易被生物降解的特性及價格昂貴的缺點 (Abumaizar & Smith 1999; Zeng *et al*. 2005)。氯化鈣 (中性鹽類) 雖然具有價格低廉、對土壤破壞程度低及對作物生長影響低小等優點，但其僅能移除土壤中可交換型態及部分酸可溶型態的鎘，移除效率較低 (Makino *et al*. 2007)。鹽酸 (強酸) 具有高鎘移除效率及價格低廉的優點，但其可能造成土壤黏土礦物的溶解 (Makino *et al*. 2008)，進而影響土壤構造的缺點。本試驗選用氯化鐵作為現地土壤清洗之試劑，此方法源自與日本農業環境技術研究所 (National Institute for Agro-Environmental Sciences; NIAES)，其團隊實驗室規模的研究結果顯示，氯化鐵同時可透過鐵在水解過程中釋放氫離子及形成 Cd-Cl 錯合物 (complexes) 兩種機制來增加土壤中鎘的溶解度，進而促進鎘的移除。研究結果亦發現，土壤經 45 mM 氯化鐵清洗後不但可降低其鎘濃度，同時也降低水稻地上部及糙米的鎘濃度，並且對於稻米的產量沒有顯著的影響，此結果指出利用氯化鐵清洗鎘汙染土壤為具有潛力的復育方法 (Makino *et al*. 2008; Makino *et al*. 2016)。然而，過去對於現地使用氯化鐵清洗土壤對於鎘移除及作物生長的研究仍不足，國內也尚未有現地土壤清洗的案例。由於實驗室試驗環境單純且容易控制，現地操作可能會受到水源供應、氯化鐵施灑及機械攪拌均勻程度、地勢高低及氯化鐵廢水排放效率等因素影響氯化鐵清洗移除鎘之效果。因此，本試驗目的為評估現地進行氯化鐵清洗法對於土壤鎘移除效率、土壤基本性質、稻作生長及水稻植株鎘累積的影響。

材料與方法

試驗田區敘述

本試驗選取 3 塊位於台中市后里區土壤含鎘之水稻田，分別稱為試驗田 A、B、C。試驗區域土壤中的鎘主要源自於鄰近工廠的廢水排入灌溉溝渠所致，試驗田 A、B、C 的土壤平均鎘濃度分別為 2.13、1.58、1.90 mg kg^{-1}。儘管皆低於環境保護署的食用作物農地土壤管制標準 (5 mg kg^{-1})，但過去都曾產出鎘濃度超過衛生福利部標準 (> 0.4 mg kg^{-1}) 的稻米。本試驗 3 塊田區面積分別約為 0.15 (試驗田 A)、0.18 (試驗田 B) 及 0.32 ha (試驗田 C)，試驗設計如下：試驗田 A 全區以氯化鐵清洗，評估氯化鐵清洗對土壤鎘移除及土壤性質的影響，以及評估氯化鐵清洗法是否造成底層土壤受到鎘汙染。為了比較有無氯化鐵清洗對稻作生長及植株鎘濃度累積的影響，將試驗田 B 和 C 分成兩部分，分別為未清洗區域及清洗區域，在氯化鐵清洗及地力回復 (施用石灰及肥料) 後，即種植水稻。

現地氯化鐵清洗

本試驗現地土壤清洗法包括 3 個主要階段，分別為氯化鐵清洗、灌溉水清洗及現地廢水處理。土壤清洗的田區周圍以 80 cm 高的塑膠板與鄰田及未清洗田區相隔，為了固定塑膠板，約將 20 cm 的塑膠隔板埋在土壤中。隔板設置的目的為避免土壤清洗產生的含重金屬廢水流出而影響鄰近環境。試驗田 A、B、C 土壤清洗的面積分別約為 0.15、0.08 及 0.15 ha。本試驗選用重金屬含量低的食品級氯化鐵來作為土壤清洗的材料，氯化鐵溶液之重量百分比為 40 %，比重約為 1.35，此材料一般用於自來水處理之混凝劑。

土壤以氯化鐵清洗前先以灌溉水將清洗區域淹水達飽和狀態，再以曳引機迴轉犁整地。接著灌水至土表以上 5 cm，再以曳引機掛載裝有氯化鐵溶液的桶子，將氯化鐵均勻施灑至清洗區域，並且以曳引機迴轉犁將土壤及氯化鐵溶液充分混合。為了比較不同氯化鐵施用量對土壤鎘移除之差異，試驗田 A、B、C 分別添加 15.15、73.13 及 21.67 Mg ha^{-1} 氯化鐵溶液，依據上述灌水量、迴轉犁的攪拌深度 (18 cm) 及土壤孔隙率 (假設為 40%) 等條件，換算田區土壤溶液中氯化鐵的濃度分別約為 30.3、146.0 及 43.3 mM。清洗後，讓田區土壤靜置過夜，隔天再將土表以上之懸浮溶液 (含鎘及氯化鐵溶液之廢水) 引至現地廢水處理設備。為了將氯化鐵清洗後田區殘留的氯化鐵溶液、鎘及其他鹽類物質移除，接著以灌溉水來清洗土壤，清洗方法與上述氯化鐵清洗步驟相同，灌溉水清洗次數為 3 次。本試驗使用的現地廢水處理設備設置於清洗區域旁，其設備包括原水槽、pH 調整槽 (快混槽及慢混槽)、凝聚劑混合槽、沉澱槽、砂濾塔、離子吸附塔及汙泥脫水機。本試驗經廢水處理設備處理後的水會再引流回清洗田區重複使用，增加水的利用效率，引流至田區的水質重金屬濃度皆低於國內灌溉水質標準。處理產生的汙泥，則依環境保護署相關規定辦理廢棄物清運及處理。由於本試驗處理後的水將引回試驗田重複使用，因此，將廢水處理過程常用的鹼液材料氫氧化鈉 (NaOH) 改成氫氧化鉀 (KOH)，避免過量的鈉排入土壤中，導致物理特性受到不利的影響。

氯化鐵清洗前後土壤採樣及基本性質分析

試驗田區在氯化鐵清洗前後皆以網格採樣方式採集表層土壤 (0–20 cm)，採樣點間隔為 10 m，分析其土壤基本性質。分析項目包含土壤 pH 值 (土水比 1 : 1 測定，McLean 1982)、電導度 (土水比 1 : 5 測定)、全氮及總碳 (元素分析儀測定)、有效性磷 (Bray No.1 萃取法，Bray & Kurtz 1945)、交換性陽離子 (醋酸銨萃取法，Thomas 1982)、陽離子交換容量 (cation exchange capacity; CEC) (1 M 醋酸銨測定法，Rhoades 1982)、質地 (比重計法，Gee & Bauder 1986)、有效性鎘濃度 (0.1 M 鹽酸萃取，Nelson *et al*. 1959) 及總鎘濃度 (微波輔助王水消化法，Lin *et al*. 2015)。此外，為了評估土壤經氯化鐵清洗後，是否會因氯化鐵清洗溶液向下滲漏而導致底層土壤受到鎘汙染，因此在土壤清洗前後，於試驗田 A 分別以土壤採樣器採集 5 個樣點之不同深度 (0–20、20–40、40–60、60–80、80–100 cm) 的土壤樣本，分析其土壤總鎘濃度

(微波輔助王水消化法，Lin *et al*. 2015)。由於重金屬於土壤的空間分布不均，上述提及之清洗前土壤採集樣點會先以全球定位系統 (global positioning system; GPS) 記錄其座標位置，清洗後再依照其座標位置採集鄰近的土壤樣本。上述土壤樣本經風乾及磨碎後，一部分土壤樣本過 0.5 mm 的篩網，此樣本用來分析土壤總有機碳、全氮及總鎘濃度；另一部分土壤樣本則過 2 mm 的篩網，此部分之土壤樣本用來分析其他土壤基本性質。

氯化鐵清洗後水稻栽種試驗

氯化鐵清洗後，3 塊試驗田土壤 pH 值介於 3–4 間，水稻種植前先以苦土石灰將土壤 pH 值調至接近清洗前之狀態 (約 pH 6.5)，石灰需要量之估算以每公頃壤土之表土增加 1 單位的 pH 值需 1.5 Mg 石灰進行估算，計算公式如下：

石灰需要量 (Mg ha^{-1})
= (目標 pH – 田間 pH) × 係數 (Mg ha^{-1})
(係數依土壤質地不同需調整，砂質壤土為 1、壤土為 1.5、粘質壤土為 2)

石灰施用量
= 石灰需要量 (Mg ha^{-1}) × 施用面積 (ha)

本試驗於試驗田 B 及 C 種植水稻 (「桃園 3 號」品種)，比較未清洗及清洗區域水稻生長及植體鎘濃度之差異。本試驗期間施用 3 次肥料，分別為種植前 3 d 施用台肥 39 號複合肥料 (12-18-12，N-P_2O_5-K_2O) 600 kg ha^{-1} (基肥)，插秧後 7 d 施用台肥 1 號複合肥料 (20-5-10，N-P_2O_5-K_2O) 270 kg ha^{-1} (第一次追肥)，以及曬田結束後施用台肥 1 號複合肥料 270 kg ha^{-1} (第二次追肥，穗肥)，共計 180 kg N ha^{-1}、135 kg P_2O_5 ha^{-1} 及 126 kg K_2O ha^{-1}。當水稻收穫時，於未土壤清洗及土壤清洗區域分別採集 5 個樣點之水稻植株及對應的根圈土壤樣本帶回實驗室進行生長指標 (地上部株高和生質量) 和植株鎘濃度之測定。稻穀產量的部分，則是於 5 個樣點各採集 20 叢水稻植株，將稻穀脫粒並放入烘箱以 70℃烘乾 48 h 後秤重。

水稻植體脫穀後，以自來水及去離子水清洗地上部，接著將稻穀及地上部樣本置入烘箱以 70℃烘乾 48 h，隨後測量其乾重。將脫殼後的糙米及地上部樣本以鈦刀磨粉機粉碎後，以硝酸-雙氧水 (10 : 1，v/v) 進行微波消化，接著以感應耦合電漿質譜儀 (Agilent 7500C, Agilent Technologies, Santa Clara, CA, USA) 分析消化液之鎘濃度。土壤樣本經風乾、磨碎及過篩後，分析土壤之 pH 值、有效性鎘濃度及總鎘濃度，土壤樣本製備及分析方法與上述相同。0.1 M 鹽酸之萃取溶液及王水消化溶液皆以感應耦合電漿光譜儀 (Ultima 2C, Horiba Jobin Yvon, Irvine, CA, USA) 分析其鎘濃度。

試驗數據處理

本試驗數據皆以平均值及試驗標準偏差方式呈現。試驗資料的統計分析先以 *t*-檢定 (*t*-test) 判定處理間效應是否達顯著水準 ($P < 0.05$)。數據呈現及 *t*-檢定皆是使用 Microsoft Excel 2010 軟體進行計算及分析。氯化鐵清洗前後之土壤鎘濃度空間分布圖，是以地理資訊系統軟體 (ArcGIS 9.3) 內的克利金 (Kriging) 內插法繪製。

結果與討論

氯化鐵清洗對土壤鎘濃度之影響

表 1 為氯化鐵清洗前後土壤有效性及總鎘濃度的變化，結果顯示，3 塊試驗田土壤有效性鎘和總鎘濃度在清洗後都有顯著降低的趨勢，試驗田 A、B、C 總鎘的移除率分別為 46.3、61.0 及 36.8%。由圖 1 氯化鐵清洗前後土壤鎘濃度空間分布的結果也發現，土壤清洗後整區土壤鎘濃度皆有下降的現象。此結果指出在本試驗操作條件下，現地氯化鐵清洗確實可降低土壤的鎘濃度，此與 Makino *et al*. (2008) 與 Makino *et al*. (2016) 的試驗結果有相同的趨勢。Makino *et al*. (2008) 與 Makino *et al*. (2016) 兩試驗土壤鎘移除率分別為 47.1% (鎘濃度由 0.690 降至 0.365 mg kg^{-1}，氯化鐵濃度為 45 mM) 和 29.4% (鎘濃度由 2.78 降至 1.96 mg kg^{-1}，氯化鐵濃度為 30 mM)，推測該試驗土壤鎘移除率和本試驗不同的原因為操作方式、土壤性質及加入的氯化鐵濃度。在氯化鐵濃度相近的條件下，Makino *et al*. (2008) 因選用土壤質地砂粒含量較高 (53.9%)

表 1. 土壤清洗處理後鎘濃度的變化。

Table 1. The change of Cd concentration in soil after soil-washing treatment.

Site	Avaiable Cd-before washing (mg kg^{-1})	Available Cd-after washing (mg kg^{-1})	Total Cd-before washing (mg kg^{-1})	Total Cd-after washing (mg kg^{-1})	Total Cd removal (%)[z]
A	1.37 ± 0.48[y]	0.88 ± 0.16***	2.13 ± 0.69	1.14 ± 0.28***	46.3
B	1.08 ± 0.24	0.50 ± 0.08***	1.58 ± 0.38	0.62 ± 0.10***	61.0
C	1.29 ± 0.61	0.87 ± 0.22**	1.90 ± 0.82	1.17 ± 0.28***	36.8

[z] Cd removal (%) = [(Total Cd-before washing – Total Cd-after washing)/Total Cd-before washing] × 100%
[y] Mean ± SD.
, * Significant at the 0.01 and 0.001 probability levels, respectively.

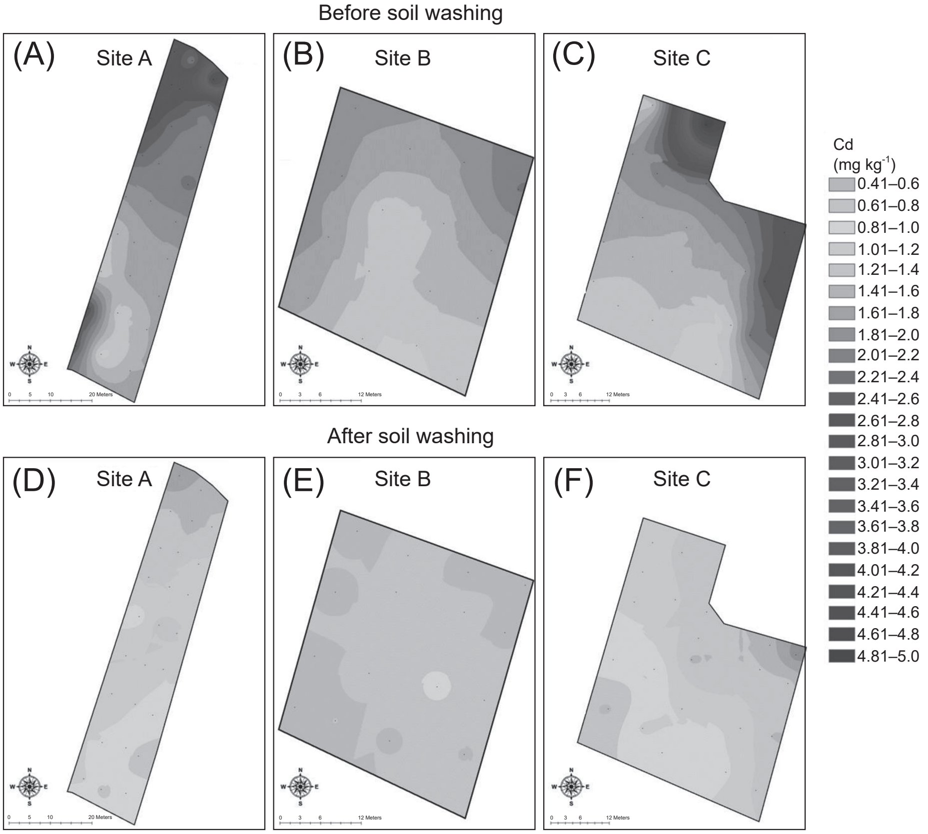

圖 1. 土壤清洗處理前後試驗田土壤鎘濃度空間分布圖。

Fig. 1. The spatial distribution of soil Cd concentration in the experimental fields before (A, B, C) and after (D, E, F) soil-washing treatment.

且於實驗室小規模操作，此有利於土壤與氯化鐵充分攪拌及含鎘廢水的排出，因此，鎘的移除效率高於本試驗田 C (砂粒含量為 42.6%)。相同地，在同樣為現地操作及氯化鐵濃度相近的條件下，相較於本試驗田 A，Makino *et al*. (2016) 之研究選用的試驗田因黏粒較高 (黏粒含量為 32.3%，土壤質地為黏質壤土)，因此鎘的移除效率也較低。由本試驗 B 的結果發現，提高氯化鐵溶液的濃度至約 150 mM 雖可增加鎘的移除率，但相較於試驗田 A 和 C 添加的氯化鐵濃度，鎘移除率增加的幅度與氯化鐵濃度不成比例，此結果表示未來可再找出理想的氯化鐵濃度及

操作方式，以同時達到提高鎘移除率且降低成本的目標。Makino *et al*. (2008) 年以氯化鐵萃取土壤鎘的試驗結果發現，當氯化鐵濃度高於 45 mM 時，對於土壤鎘的移除率影響不大，因此認為在該試驗的條件下，氯化鐵濃度 45 mM 為土壤清洗理想的濃度。本試驗結果也觀察到，理想的氯化鐵濃度亦會受土壤性質不同而異。除了上述提及的土壤性質及操作條件會影響土壤的鎘移除外，本試驗發現，田區的形狀亦會影響土壤清洗的效率。由試驗田 A 和 C 的結果顯示，儘管試驗田 A 的氯化鐵施用量低於試驗田 C，但鎘的移除率卻較高，推測這是由於試驗田 A 的形狀方正所致 (圖 1)，此有利於土壤清洗時農機的操作，使土壤和氯化鐵溶液能夠充分攪拌均勻，進而提高鎘的移除率 (表 1)。

表 1 顯示，3 塊試驗田土壤清洗後有效性鎘 (0.1 M HCl 萃取) 皆有顯著的下降，其降低的鎘含量占總鎘降低量的 49.5–60.4%，其餘降低的鎘為溶解度較低的型態。此型態鎘降低的原因，推測是透過田間廢水排放時，將部分粒徑小且懸浮於廢水中的黏粒排出所致。然而，Makino *et al*. (2008) 的試驗結果顯示，0.1 M HCl 萃取的鎘降低的含量占總鎘降低量的 95.7%，此明顯高於本試驗結果。這主要是可能因為該研究使用的土壤鎘有效性較高所致，該土壤 0.1 M HCl 抽出鎘濃度占總鎘量的比值為 92.0%，本試驗土壤則僅占 64.3–68.4%。由此得知，土壤中鎘的鍵結能力也會影響氯化鐵清洗移除鎘的效率。此外，Makino *et al*. (2007) 於現地進行氯化鈣 (100 mM) 清洗鎘汙染土壤，試驗結果指出土壤鎘鎘濃度僅降低 17%。此移除率明顯較本試驗低，兩者的差別在於清洗溶液的鎘萃取的機制。氯化鈣是利用鈣離子與吸附在土壤中的鎘進行離子交換，接著使溶液中的鎘離子與氯離子形成穩定性高且不易被土壤吸附的 Cd-Cl 錯合物，進而導致鎘的移除 (Makino *et al*. 2007)；氯化鐵除了可透過氯離子錯合的方式移除土壤鎘以外，也可以利用鐵在水解過程中釋放的氫離子來增加鎘的溶解度 (Makino *et al*. 2006)，兩種機制共同增加鎘的移除效率。儘管氯化鈣清洗對土壤的破壞性較氯化鐵低，但考量鎘移除效率，氯化鐵仍較適合用來當作清洗鎘汙染土壤的材料。

圖 2 為氯化鐵清洗前後，試驗田 A 不同深度土壤鎘濃度的變化。結果顯示，土壤清洗後 0–20 及 20–40 cm 兩層的土壤平均鎘濃度有下降的趨勢，這是因為土壤中的鎘因氯化鐵清洗移除所致。然而，相較於清洗前，在深度 40–60、60–80、80–100 cm 三層的土壤鎘濃度在清洗後都沒有增加的趨勢，這代表本試驗操作條件下，表層土壤的氯化鐵溶液不會滲漏至底層土壤。此結果也證明，本試驗田的犁底層確實可達到阻隔氯化鐵溶液向下滲漏的功能，避免底層土壤及地下水在土壤清洗後受到鎘的汙染。此外，由本試驗廢水處理水質分析結果指出，除了電導度值及氯離子濃度高於我國的灌溉水質標準外，其餘分析之重金屬離子 (鎘、鉻、銅、鋅、鎳、鉛、砷、汞)、硫酸鹽及總氮濃度皆符合標準，其中電導度值提高主要原因為溶液中的氯離子濃度較高所致 (數據未呈現)。Nagai *et al*. (2012) 於現地氯化鐵清洗和 Makino *et al*. (2007) 於現地氯化鈣清洗產生之廢水經處理後，水質分析結果同樣也顯示氯離子的濃度仍偏高，這表示若要現地快速處理廢水中的氯離子是相當困難的。

氯化鐵清洗對土壤性質之影響

過去研究指出，土壤清洗法可有效降低土

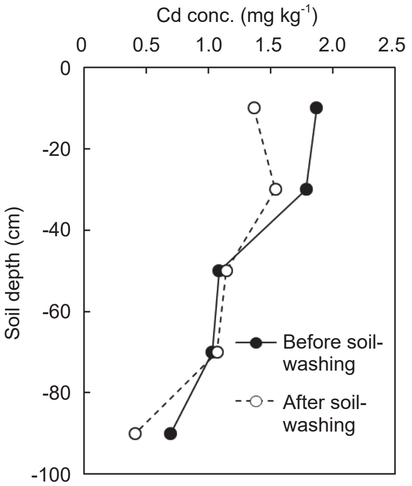

圖 2. 土壤清洗處理後試驗田 A 不同深度土壤鎘濃度之變化。

Fig. 2. The change of Cd concentration in site A soils of different depths after soil-washing treatment.

壤中的鎘濃度，但使用藥劑的種類可能影響土壤的性質。例如使用 EDTA 不易被生物降解，易造成環境負擔 (Zeng *et al*. 2005)；使用過量的鹽酸溶液可能導致土壤中黏土礦物的溶解，不利於後續的地力回復 (Ogawa *et al*. 1985)。表 2 為本試驗氯化鐵清洗前後土壤性質的變化，結果顯示，3 塊試驗田土壤 pH 值在清洗後有都有顯著的下降。其中，以氯化鐵施用量最高的試驗田 B 下降的程度最大，這也導致試驗田 B 土壤有效性磷及交換性鈣和鎂降低的程度大於其他兩塊試驗田。上述問題可藉由施用苦土石灰同時提高土壤 pH 值及補充土壤中的交換性鈣及鎂，另外可透過肥料的施用來補足土壤中的有效性磷及其他養分。土壤電導度 (electrical conductivity; EC) 值在清洗後有增加的現象，其中也以試驗田 B 增加的幅度最大，這是由於氯化鐵中的氯離子殘留和土壤 pH 下降導致土壤吸附的陽離子釋出增加所致。儘管如此，本試驗導致的電導度值增加對於水稻生長及稻穀產量無顯著的影響 (圖 3)，其原因將於下一節次說明。在土壤的總氮、總碳、CEC 及質地的部分，則是清洗前後無顯著差異。本試驗氯化鐵鐵清洗前後土壤性質變化之趨勢與 Makino *et al*. (2008) 之研究結果相似。雖然清洗後對於土壤肥力有些微的影響，但此可透過石灰及肥料的施用達到地力的回復。此外，影響土壤保肥能力指標的有機質 (總碳) 及 CEC 在清洗後無顯著變化，代表本試驗氯化鐵清洗對土壤的破壞程度低，這也表示未來再補充養分即可使土壤恢復可耕種的狀態。Makino *et al*. (2008) 也透過氯化鐵清洗後僅有低於 1% 的鋁被溶解釋放，以此證明氯化鐵清洗不會對土壤的黏土礦物造成嚴重的破壞。

氯化鐵清洗對於稻作生長之影響

由表 2 結果證實，氯化鐵清洗對土壤的破壞程度不高，本節將進一步比較土壤有無氯化鐵清洗對水稻株高、地上部生質量及稻穀產量的影響 (圖 3)。試驗結果顯示，相較於未清洗土壤，兩塊試驗田氯化鐵清洗後，水稻株高、地上部生質量及稻穀產量統計上均無呈現顯著下降的趨勢。此結果指出，現地氯化鐵清洗土壤對於後續稻作的生長無顯著影響，這也呼應前一節土壤基本性質的分析結果，清洗後透過石

表 2. 土壤清洗處理後基本性質的變化。
Table 2. The change of soil basic properties after soil-washing treatment.

Basic property[z]	Site A		Site B		Site C	
	Before	After	Before	After	Before	After
pH	6.630 ± 0.240[y]	4.700 ± 0.290***	6.320 ± 0.210	3.040 ± 0.140***	6.110 ± 0.500	4.090 ± 0.420***
EC (μS cm^{-1})	116.000 ± 31.200	281.000 ± 41.200***	114.000 ± 40.200	666.000 ± 116.000***	134.000 ± 36.800	443.000 ± 113.000***
Total N (%)	0.078 ± 0.007	0.089 ± 0.007***	0.069 ± 0.006	0.0075 ± 0.007*	0.077 ± 0.010	0.073 ± 0.003
Total C (%)	0.800 ± 0.080	0.850 ± 0.060*	0.750 ± 0.070	0.740 ± 0.060	0.860 ± 0.210	0.770 ± 0.040
Available P (mg kg^{-1})	6.360 ± 2.360	4.660 ± 1.700*	8.920 ± 2.570	3.570 ± 2.020***	12.100 ± 3.720	7.370 ± 3.420***
Exchangeable Ca (mg kg^{-1})	727.000 ± 126.000	466.000 ± 49.500***	655.000 ± 61.800	362.000 ± 55.900***	726.000 ± 191.000	482.000 ± 70.000***
Exchangeable Mg (mg kg^{-1})	133.000 ± 13.500	93.500 ± 9.380***	131.000 ± 10.300	78.700 ± 12.500***	120.000 ± 19.400	99.400 ± 11.600***
CEC (cmol kg^{-1})	6.410 ± 0.670	6.030 ± 0.460*	6.240 ± 0.800	6.220 ± 0.810	6.360 ± 0.740	6.300 ± 0.390
Texture	Loam	Loam	Loam	Loam	Loam	Loam

[z] EC: electrical conductivity; N: nitrogen; C: carbon; P: phosphorus; CEC: cation exchange capacity.
[y] Mean ± SD.
*, **, *** Significant at the 0.5, 0.01 and 0.001 probability levels, respectively.

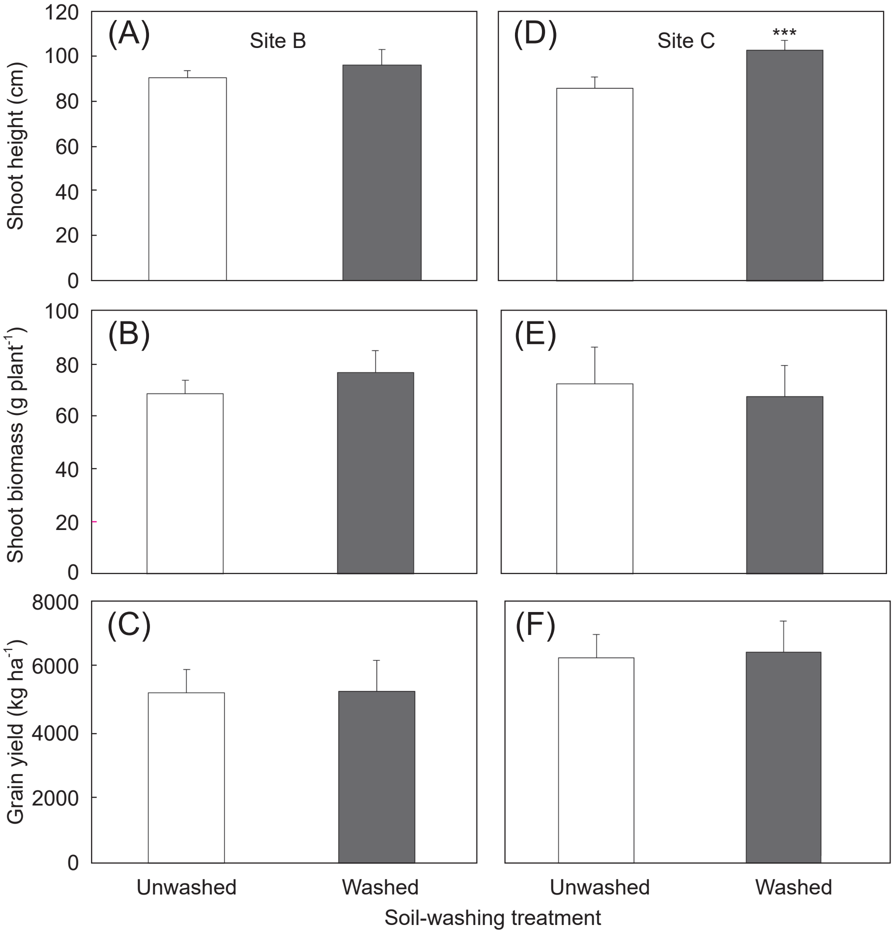

圖 3. 土壤清洗處理對水稻株高、地上部生質量及稻穀產量的影響。

Fig. 3. The effects of soil-washing treatment on the shoot height (A, D), shoot biomass (B, E), and grain yield (C, F) of paddy rice. ***significant at 0.001 probability level.

灰調整 pH 值及肥料補足養分即可讓稻作正常生長。Makino *et al*. (2008) 的研究結果同樣顯示，氯化鐵清洗確實不會導致稻作的生長受阻，反而發現在氯化鐵清洗後水稻地上部生質量及產量都有略高於未清洗的土壤。水稻被分類為對鹽類耐受度中度敏感 (土壤電導度耐受範圍為飽和抽出液電導度 2,000–4,000 μS cm^{-1}) 的作物 (Maas & Grattan 1999)，對照本試驗田 B 和 C 清洗後土壤之電導度值 (土水比 1 : 5) 分別為 666 及 443 μS cm^{-1} (表 2)，換算飽和抽出液電導度分別約為 3,330 及 2,215 μS cm^{-1}，此值仍低於水稻鹽害的臨界值。由此得知，在本試驗操作條件及氯化鐵添加濃度範圍內 (< 150 mM)，土壤清洗後仍可正常種植水稻，但後續若要種植鹽害敏感之作物 (土壤電導度耐受範圍 < 2,000 μS cm^{-1})，則須將土壤改良後才可進行。

氯化鐵清洗對於植株鎘濃度之影響

由稻作生長結果指出，氯化鐵清洗對於水稻的生長及產量沒有顯著的影響，接著則是比較土壤有無氯化鐵清洗對植體鎘濃度之影響。試驗結果顯示，種植於經氯化鐵清洗與未清洗之水稻地上部及糙米鎘濃度統計上無顯著差異，但平均值都有下降的趨勢，這主要是因為田區土壤鎘濃度的空間變異所導致 (表 3)。試驗田 B 及 C 水稻地上部分別降低 9.8% 及 41.7%，糙米

表 3. 土壤清洗處理對土壤及水稻植株鎘濃度的影響。

Table 3. The effects of soil-washing treatment on the soil pH and the concentration of Cd in soils and rice plants.

	Site B		Site C	
Analytical item	Unwashed	Washed	Unwashed	Washed
Soil pH	6.95 ± 0.08[z]	6.16 ± 0.42**	6.90 ± 0.16	6.69 ± 0.14
Available-Cd in soil (mg kg^{-1})	0.79 ± 0.11	0.35 ± 0.08***	0.77 ± 0.04	0.72 ± 0.07
Total-Cd in soil (mg kg^{-1})	1.03 ± 0.11	0.49 ± 0.05***	0.99 ± 0.11	0.91 ± 0.14
Shoot-Cd (mg kg^{-1})	2.76 ± 1.20	2.49 ± 0.70	5.73 ± 2.10	3.34 ± 1.17
Unpolished grain-Cd (mg kg^{-1})	0.60 ± 0.20	0.51 ± 0.11	1.07 ± 0.37	0.71 ± 0.17

[z] Mean ± SD.

, * Significant at the 0.01 and 0.001 probability levels, respectively.

分別降低 15% 及 33.6%，由土壤有效性鎘濃度及總鎘濃度也顯示土壤清洗後有較低的趨勢。相較兩試驗田，試驗田 B 清洗後有效性鎘濃度及總鎘濃度皆低於試驗田 C，糙米及地上部累積的鎘濃度也低於試驗田 C。因此，本試驗結果指出，土壤經氯化鐵清洗後確實可同時降低土壤及水稻植體的鎘濃度，此與 Makino *et al*. (2008) 的研究結果有相同的趨勢。此外，本試驗結果亦發現，有無清洗間土壤鎘濃度差異程度則是試驗田 B 大於試驗田 C，此與植株鎘濃度的趨勢相反。雖然試驗田 B 清洗後土壤鎘濃度顯著降低，但其糙米鎘濃度降低程度低於試驗田 C，這主要是因為試驗田 B 土壤經清洗後土壤 pH 值較低以及土壤氯離子濃度較高所致。儘管土壤清洗後有添加石灰提升 pH 值，但由於試驗田 B 添加較高濃度的氯化鐵，土壤 pH 值無法馬上恢復至清洗前的狀態，進而提升鎘的有效性。由表 2 土壤清洗後的電導度值顯示，試驗田 B 清洗後明顯高於試驗田 C，這也代表土壤殘留的氯離子濃度也較高，由於土壤溶液中的氯離子與鎘離子易形成穩定性高且不易被土壤吸附的 Cd-Cl 錯合物，使鎘的移動性增加，進而增加土壤中鎘的生物有效性 (Bolan *et al*. 1999)。Smolders & McLaughlin (1996) 的研究結果亦顯示，高濃度的氯離子與鎘形成的 $Cd\text{-}Cl^{+}$ 錯合物，容易被植物根部吸收。由此可見，氯化鐵清洗後的土壤 pH 及氯離子濃度的調整為降低水稻植株鎘累積的重要步驟，可透過多次以灌溉水清洗來降低土壤氯離子的殘留，搭配增加石灰施用後與土壤反應的時間 (提升土壤 pH 值)，方可降低糙米鎘的累積。此外，本試驗區域為灌溉水缺乏的區域，5.5 d 輪灌 1 次，水稻生長期間常呈現田間水分不足的狀態，在此狀態下鎘以溶解度高的硫酸鎘 ($CdSO_4$) 型態存在。因此，導致試驗田 B 及 C 土壤清洗後，儘管土壤鎘濃度降低，糙米的鎘濃度仍超過我國衛生福利部的限量標準 (0.4 mg kg^{-1})。

結論

總結本試驗結果，氯化鐵清洗確實可有效降低土壤的鎘濃度，對土壤物理和化學特性破壞程度低，並且對水稻生長及稻穀產量無顯著影響。然而，由於稻穀中鎘的累積除了受土壤鎘濃度影響外，同時也受到土壤 pH、氯離子濃度及水分條件等因素所影響，故土壤經氯化鐵清洗降低鎘濃度後，仍需搭配合適的地力回復及土壤管理 (提高土壤 pH 值、降低土壤氯離子濃度及湛水管理)，方可降低糙米鎘濃度超標的風險。因此，本試驗結果評估現地氯化鐵清洗法對於移除農地土壤鎘為具潛力且環境友善的復育技術。

誌謝

本研究承行 2014 年農業試驗所與環境保護署合辦「農地鎘潛在危害地區農田管理試辦計畫」及 2014 年農糧署「農作物汙染監測管制及損害查處計畫 (03 農糧-3.1-資-02)」經費挹注，謹此表達感謝。

引用文獻

Abumaizar, R. J. and E. H. Smith. 1999. Heavy metal contaminants removal by soil washing. J. Hazard. Mater. 70:71–86.

Adamu, C. I. and T. N. Nganje. 2010. Heavy metal contamination of surface soil in relationship to land use patterns: A case study of Benue State, Nigeria. Mater. Sci. Appl. 1:127–134.

Arao, T., A. Kawasaki, K. Baba, S. Mori, and S. Matsumoto. 2009. Effects of water management on cadmium and arsenic accumulation and dimethylarsinic acid concentrations in Japanese rice. Environ. Sci. Technol. 43:9361–9367.

Basta, N. T. and S. L. McGowen. 2004. Evaluation of chemical immobilization treatments for reducing heavy metal transport in a smelter-contaminated soil. Environ. Pollut. 127:73–82.

Bolan, N. S., D. C. Adriano, P. Duraisamy, A. Mani, and K. Arulmozhiselvan. 2003a. Immobilization and phytoavailability of cadmium in variable charge soils. I. Effect of phosphate addition. Plant Soil 250:83–94.

Bolan, N. S., D. C. Adriano, and P. Duraisamy. 2003b. Immobilization and phytoavailability of cadmium in variable charge soils. II. Effect of lime addition. Plant Soil 251:187–198.

Bolan, N. S., R. Naidu, M. A. R. Khan, R. W. Tillman, and J. K. Syres. 1999. The effects of anion sorption on sorption and leaching of cadmium. Aust. J. Soil Res. 37:445–460.

Bolan, N. S., T. Makino, A. Kunhikrishnan, P. J. Kim, S. Ishikawa, M. Murakami, R. Naidu, and M. B. Kirkham. 2013. Chapter 4: Cadmium contamination and its risk management in rice ecosystems. p.183–273. *in*: Advances in Agronomy. Vol. 119. (Sparks, D. L., ed.) Academic Press. Amsterdam, The Netherlands. 476 pp.

Bray, R. H. and L. T. Kurtz. 1945. Determination of total, organic, and available forms of phosphorus in soils. Soil Sci. 59:39–46.

Figueroa, E. 2008. Are more restrictive food cadmium standards justifiable health safety measures or opportunistic barriers to trade? An answer from economics and public health. Sci. Total Environ. 389:1–9.

Gee, G. W. and J. W. Bauder. 1986. Particle-size analysis. p.383–411. *in*: Methods of Soil Analysis. Part 1-Physical and Mineralogical Methods. 2nd ed. (Klute, A., ed.). ASA, SSSA. Madison, WI. 1188 pp.

Godt, J., F. Scheidig, C. Grosse-Siestrup, V. Esche, P. Brandenburg, A. Reich, and D. A. Groneberg. 2006. The toxicity of cadmium and resulting hazards for human health. J. Occup. Med. Toxicol. 1:22.

Hong, C. O., D. K. Lee, and P. J. Kim. 2008. Feasibility of phosphate fertilizer to immobilize cadmium in a field. Chemosphere 70:2009–2015.

Hong, C. O., D. Y. Chung, D. K. Lee, and P. J. Kim. 2010. Comparison of phosphate materials for immobilizing cadmium in soil. Arch. Environ. Contam. Toxicol. 58:268–274.

Hu, P. J., Z. Li, C. Yuan, Y. N. Ouyang, L. Q. Zhou, J. X. Huang, Y. J. Huang, Y. M. Luo, P. Christie, and L. H. Wu. 2013. Effect of water management on cadmium and arsenic accumulation by rice (*Oryza sativa* L.) with different metal accumulation capacities. J. Soils Sediments 13:916–924.

Kato, M., S. Ishikawa, K. Inagaki, K. Chiba, H. Hayashi, S. Yanagisawa, and T. Yoneyama. 2010. Possible chemical forms of cadmium and varietal differences in cadmium concentrations in the phloem sap of rice plants (*Oryza sativa* L.). Soil Sci. Plant Nutr. 56:839–847.

Kuo, S., B. Huang, and R. Bembenek. 2007. Release of cadmium from a triple superphosphate and a phosphate rock in soil. Soil Sci. 172:257–265.

Lin, Y. W., T. S. Liu, H. Y. Guo, C. M. Chiang, H. J. Tang, H. T. Chen, and J. H. Chen. 2015. Relationships between Cd concentrations in different vegetables and those in arable soils, and food safety evaluation of vegetables in Taiwan. Soil Sci. Plant Nutr. 61:983–998.

Loganathan, P., M. J. Hedley, and N. D. Grace. 2008. Pasture soils contaminated with fertilizer-derived cadmium and fluorine: Livestock effects. Rev. Environ. Contam. Toxicol. 192:29–66.

Maas, E. V. and S. R. Grattan. 1999. Crop yields as affected by salinity. p.55–108. *in*: Agricultural Drainage, Agronomy Monograph No. 38. (Skaggs, R. W. and J. van Schilfgaarde, eds.) ASA, CSSA and SSSA. Madison, WI. 1328 pp.

Makino, T., K. Sugahara, Y. Sakurai, H. Takano, T. Kamiya, K. Sasaki, T. Itou, and N. Sekiya. 2006. Remediation of cadmium contamination in paddy soils by washing with chemicals: Selection of washing chemicals. Environ. Pollut. 144:2–10.

Makino, T., H. Takano, T. Kamiya, T. Itou, N. Sekiya, M. Inahara, and Y. Sakurai. 2008. Restoration of cadmium-contaminated paddy soils by washing with ferric chloride: Cd extraction mechanism and bench-scale verification. Chemosphere 70:1035–1043.

Makino, T., T. Kamiya, H. Takano, T. Itou, N. Sekiya, K. Sasaki, Y. Maejima, and K. Sugahara. 2007. Remediation of cadmium-contaminated paddy soils by washing with calcium chloride: Verification of on-site washing. Environ. Pollut. 147:112–119.

Makino, T., Y. Maejima, I. Akahane, T. Kamiya, H. Takano, S. Fujitomi, T. Ibaraki, A. Kunhikrishnan, and N. Bolan. 2016. A practical soil washing method for use in a Cd-contaminated paddy field, with simple on-site wastewater treatment. Geoderma 270:3–9.

McLean, E. O. 1982. Soil pH and lime requirement. p.199–224. *in*: Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. (Page, A. L., R. H. Miller, and D. R. Keeney, eds.) ASA, SSSA. Madison, WI. 1159 pp.

Nagai, T., T. Horio, A. Yokoyama, T. Kamiya, H. Takano, and T. Makino. 2012. Ecological risk assessment of on-site soil washing with iron(III) chloride in cadmium-contaminated paddy field. Ecotox. Environ. Safe. 80:84–90.

Nelson, J. L., L. C. Boawn, and F. G. Viets. 1959. Method for assessing Zinc status of soils using acid-extractable Zinc and "titratable alkalinity" values. Soil Sci. 88:275–283.

Ogawa, B., K. Taguchi, and R. Uemura. 1985. Studies on the mitigation of damage for crop by heavy metal in soils. Bull. Akita Agric. Exp. Stn. 27:1–28. (in Japanese with English abstract)

Rhoades, J. D. 1982. Cation exchange capacity. p.149–157. *in*: Methods of soil analysis. Part 2. Chemical and microbiological Properties. (Page, A. L. R. H. Miller, and D. R. Keeney, eds.) ASA, SSSA. Madison, WI. 1159 pp.

Satarug, S., J. R. Baker, S. Urbenjapol, M. Haswell-Elkins, P. E. B. Reilly, D. J. Williams, and M. R. Moore. 2003. A global perspective on cadmium pollution and toxicity in non-occupationally exposed population. Toxicol. Lett. 137:65–83.

Schoeters, G., E. Den Hond, M. Zuurbier, R. Naginiene, P. van den Hazel, N. Stilianakis, R. Ronchetti, and J. G. Koppe. 2006. Cadmium and children: Exposure and health effects. Acta. Paediatr. 95:50–54.

Smolders, E. and M. J. Mclaughlin. 1996. Chloride increases cadmium uptake in Swiss chard in a resin-buffered nutrient solution. Soil. Sci. Soc. Amer. J. 60:1443–1447.

Sun, Y. B., Y. Li, Y. M. Xu, X. F. Liang, and L. Wang. 2015. In situ stabilization remediation of cadmium (Cd) and lead (Pb) co-contaminated paddy soil using bentonite. Appl. Clay Sci. 105:200–206.

Thomas, G. W. 1982. Exchangeable cations. p.159–165. *in*: Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties (Page, A. L., R. H. Miller, and D. R. Keeney, eds.) ASA, SSSA, Madison, WI. 1159 pp.

Zeng, Q. R., S. Sauvé, H. E. Allen, and W. H. Hendershot. 2005. Recycling EDTA solutions used to remediate metal-polluted soils. Environ. Pollut. 133:225–231.

Assessing the Effects of Soil Washing with Ferric Chloride on Cadmium Removal, Soil Properties and Rice Growth in Cd-containing Paddy Fields

Chien-Hui Syu[1,*], Yu-Wen Lin[2], Chia-Yi Cheng[3], Ya-Ting Ho[3], Chu-Chung Chen[1], Tsang-Sen Liu[2], and Horng-Yuh Guo[4]

Abstract

Syu, C. H., Y. W. Lin, C. Y. Cheng, Y. T. Ho, C. C. Chen, T. S. Liu, and H. Y. Guo. 2018. Assessing the effects of soil washing with ferric chloride on cadmium removal, soil properties and rice growth in Cd-containing paddy fields. J. Taiwan Agric. Res. 67(4):365–376.

Cadmium (Cd) polluted in paddy field is the serious problem, and rice consumption is the major exposure pathway for Cd in many Asia area, thereby reducing Cd uptake by rice from soils is very important for lowering the exposure risk of Cd to human. Soil washing is recognized as a high efficient remedial technology for Cd removal. However, limited information is available for assessing the Cd removal and crop growth after on-site soil washing. Therefore, the objective of this study is to evaluate the effects of on-site soil washing with ferric chloride ($FeCl_3$) on the Cd removal, soil properties, and rice growth in paddy fields. Three Cd-containing paddy fields were selected for this study (Cd concentration in soil of fields A, B, and C are 2.13, 1.58, and 1.90 mg kg^{-1}, respectively), which located in Houli Dist., Taichung City, Taiwan. The Cd concentrations and soil properties were measured before and after soil washing, and the soil samples were collected by grid sampling method. In addition, it also measured the growth indices and Cd concentrations in plant tissues of paddy rice grown in tested field after soil washing. The results indicated that the average Cd concentrations in soils of three tested fields were significant decreased after soil washing with $FeCl_3$, and the extents of decrease in Cd concentrations of tested fields were 36.8, 46.3, and 61.0%, respectively. It observed that the pH, electrical conductivity (EC) and available phosphorus (P) of soils were changed after soil washing, but there were no significant differences in total nitrogen (N), organic carbon (C), and cation exchange capacity (CEC) of soils between before and after soil washing. From the results of rice growth, compared with control treatment, it indicated that there was no significant decrease in shoot height, shoot biomass and grain yield of paddy rice grown in soils after soil washing. It also found that the concentration of Cd accumulated in rice plants were reduced. In conclusion, the results of this study indicated that the on-site soil washing with $FeCl_3$ could reduce the Cd concentration in soils effectively, and it has no significant effect on the plant growth and grain yield of paddy rice. In spite of the soil washing had slightly effect on the soil properties, it could be restored through lime and fertilizer supplied. Therefore, it suggests that the on-site soil washing with $FeCl_3$ may be a feasible remedial technology for Cd removal in Cd-contaminated paddy soils.

Key words: Cadmium, Soil washing, Ferric chloride, Paddy rice, On-site trial.

Received: May 2, 2018; Accepted: June 1, 2018.
[*] Corresponding author, e-mail: chsyu@tari.gov.tw
[1] Assistant Research Fellows, Agricultural Chemistry Division, Taiwan Agricultural Research Institute, Taichung, Taiwan, ROC.
[2] Associate Research Fellows, Agricultural Chemistry Division, Taiwan Agricultural Research Institute, Taichung, Taiwan, ROC.
[3] Research Assistants, Agricultural Chemistry Division, Taiwan Agricultural Research Institute, Taichung, Taiwan, ROC.
[4] Research Fellow and Director, Agricultural Chemistry Division, Taiwan Agricultural Research Institute, Taichung, Taiwan, ROC.

台灣農業研究 (J. Taiwan Agric. Res.) 67(4):377–386 (2018)
DOI:10.6156/JTAR.201812_67(4).0006 研究報告

利用中和亞磷酸溶液防治馬鈴薯青枯病

林靜宜 [1,*] 林慧如 [2]

摘要

林靜宜、林慧如。2018。利用中和亞磷酸溶液防治馬鈴薯青枯病。台灣農業研究 67(4):377–386。

由青枯病菌 (*Ralstonia solanacearum*) 引起之細菌性萎凋病為世界馬鈴薯生產栽培之限制因子之一，其防治相當困難，目前尚未有合適之化學藥劑可供防治，僅能以健康種薯、土壤處理及輪作等方式加以管理。本研究利用亞磷酸與氫氧化鉀以 1 : 1 (w/w) 方式配製成亞磷酸-氫氧化鉀中和液 (neutralized phosphoric acid solution; NPA) 後，於溫室中評估不同施用方法，連續施用 4 次後對馬鈴薯青枯病之防治效果。結果發現當 NPA 濃度為 0.08% 時，以土壤澆灌法處理之植株其罹病度為 40%，而利用葉片噴施法處理為 92%，顯示土壤澆灌法使用亞磷酸溶液的防治效果較葉片噴施法更能降低罹病度。進一步於溫室試驗中利用土壤澆灌法試驗不同濃度 NPA 對青枯病之防治效果，結果顯示使用 0.05–2.00% NPA 均可降低馬鈴薯青枯病之罹病度，其中以高濃度 1% 及 2% 效果最佳，罹病度皆為 0%；其次則為中濃度之 0.1–0.5% NPA，罹病度為 36.67–46.67%，低濃度 0.05% NPA 防治效果較不顯著，罹病度為 83.33%。然而，由於施用高濃度 2% NPA 時，會導致馬鈴薯植株生長出現矮化及地下部無法結薯的情形，故建議使用濃度不宜超過 1%，以避免藥害產生。目前利用 NPA 防治細菌性病害之作用機制尚未釐清，藉由本研究體外試驗發現，NPA 具抑制青枯病菌繁殖速度之效果，隨著亞磷酸濃度增加，抑制效果越顯著，惟仍無法完全抑制其繁殖及直接殺死病原細菌。

關鍵詞：亞磷酸、馬鈴薯、青枯病。

前言

馬鈴薯 (*Solanum tuberosum* L.) 屬茄科一年生草本植物，其塊莖富含澱粉、蛋白質、礦物質及維生素等養分，為世界重要糧食作物。在台灣，馬鈴薯以中南部為主要產區，普遍於秋、冬季裡栽種。根據 2016 年農業委員會農業統計年報 (http://agrstat.coa.gov.tw/sdweb/public/official/OfficialInformation.aspx) 顯示，總種植面積為 2,724 ha，其中雲林縣栽培面積最大，其次分別為嘉義縣、台中市及台南市等地區。目前栽培品種主要有「克尼伯」(大葉種) 及「大西洋」等，其中以兼具鮮食與加工特性的「克尼伯」栽培面積最廣。因台灣氣候環境高溫多濕，有利於病蟲害孳生危害而影響馬鈴薯生長，其威脅馬鈴薯生產的重要限制因子之一，係由青枯病菌 (*Ralstonia solanacearum*) 所引起之細菌性萎凋病或褐腐病 (brown rot)。病菌往往造成系統性感染，使馬鈴薯植株表現葉片失水、萎凋及維管束褐化等病徵，最終導致作物死亡以及嚴重的經濟損失 (Hayward 1991; Elphinstone 2005; Denny 2006)。

R. solanacearum 寄主範圍廣泛，可危害 54 科 450 種以上的寄主植物 (Denny 2006; Wicker *et al*. 2007)。青枯病菌為一複合種 (species complex)，由許多不同的菌系組成，在生理特性、遺傳表現及寄主範圍等方面具有豐富的多樣性 (Hayward 2000; Genin & Boucher

投稿日期：2018 年 4 月 17 日；接受日期：2018 年 6 月 4 日。
[*] 通訊作者：eris2024@dns.caes.gov.tw
[1] 農委會農業試驗所嘉義農業試驗分所植物保護系助理研究員。台灣 嘉義市。
[2] 農委會農業試驗所嘉義農業試驗分所植物保護系研究助理。台灣 嘉義市。

2004; Fegan & Prior 2005)。目前已發現可感染台灣之茄科作物的 *R. solanacearum* 菌系有寄主範圍較廣之第一生理小種 (Hsu 1991; Lin 2008)，以及主要對馬鈴薯、番茄具有強病原性的第三生理小種 (Chiou 2002; Wu *et al*. 2010; Wu *et al*. 2011)。其中，*R. solanacearum* 第三生理小種於冷涼環境容易大發生，此菌系所造成之馬鈴薯青枯病，又稱為褐腐病，罹病株地上部出現萎凋病徵，地下部薯塊 (tuber) 則出現褐腐徵狀，已成為嚴重危害溫帶地區馬鈴薯的青枯病菌 (Ciampi & Sequeira 1980)。台灣於 1999 年冬天在台中市神岡、后里地區首次發現 *R. solanacearum* 第三生理小種危害馬鈴薯，2006 年底至 2007 年 1 月間亦於雲林縣斗南及虎尾馬鈴薯產區造成大面積的萎凋病徵及經濟損失 (Chiou 2002; Wu *et al*. 2010; Wu *et al*. 2011)。

目前已有許多研究報導顯示，亞磷酸 (phosphorous acid; H_3PO_3) 對卵菌類引起之病害 (Oomycetes diseases) 具有良好的防治效果 (Cohen & Coffey 1986; Ann 2001; Johnson *et al*. 2004)，其中以對各種作物之疫病研究最為廣泛且深入。近年來雖有研究證實可利用亞磷酸化合物來防治細菌性病害 (Norman *et al*. 2006; Wen *et al*. 2007; Wen *et al*. 2009; Lin & Wang 2011)，但相關研究報導並不多。此外，亞磷酸為強酸，水溶液的酸鹼值約為 pH 2.0–3.0，不適宜直接使用，需與鹼性物質中和至酸鹼值為 pH 5.5–6.5 後，才可施用於植物體。行政院農業委員會農業試驗所已研發出一種便捷的亞磷酸溶液配製法 (Ann *et al*. 2000)，並已技術轉移 (農試所技術轉移案 No.9，2006) 至業者，使用者可自行配製。然而，亞磷酸溶於水後易氧化成磷酸而失去功效，因此最好配製後當日使用。

國內已廣泛應用亞磷酸來防治作物的疫病、晚疫病及白粉病等真菌病害 (Ann 2001)，卻少有應用在防治細菌性病害之研究報導。本研究即是探討中和後之亞磷酸溶液，以不同施用方式及不同濃度使用後對馬鈴薯青枯病之防治效果，以瞭解亞磷酸對青枯病發生之影響，並評估其防治應用之可行性。

材料與方法

試驗菌株來源與接種原製備

本研究使用之青枯病 (*Ralstonia solanacearum*) 菌株為 RS1118 (第三生理小種，第二生化型)，係分離自嘉義市罹患青枯病之馬鈴薯植株 (Chuang *et al*. 2015)，並以甘油保存於 -80℃供日後試驗使用。將保存於甘油的青枯病菌利用滅菌後之移植環畫線培養於 Triphenyl tetrazolium chloride (TTC) 培養基 [1 L 培養基中含 1 g 酪蛋白 (casein hydrolysate)，10 g 蛋白腖 (peptone)，5 g 葡萄糖 (glucose)，20 g 瓊脂 (agar)，經高溫滅菌後再加入 5 mL 之 1% TTC] (Kelman 1954)，再挑取單一菌落於 Yeast extract dextrose calcium carbonate (YDC) 培養基 [1 L 培養基中含 10 g 酵母抽出物 (yeast extract)，20 g 葡萄糖 (dextrose)，20 g 碳酸鈣 (calcium carbonate) 及 15 g 瓊脂] (Wilson *et al*. 1967)。經 48 h 增量培養後，以無菌水洗出成為細菌懸浮液。懸浮液利用光電比色計於 600 nm 波長下測定，調整其吸光值 (optical density; OD) 為 0.3，菌量濃度約為 10^8 CFU mL^{-1} 即為接種源。

亞磷酸-氫氧化鉀中和液 (neutralized phosphoric acid solution; NPA) 之製備

試驗使用之亞磷酸 (99% H_3PO_3) (禾康肥料有限公司，台灣台中市) 為白色透明結晶體，中和用之氫氧化鉀 (95.5% KOH) (禾康肥料有限公司，台灣台中市) 為白色薄片狀之產品。於配製溶液前，先依所需濃度 (0.05、0.08、0.1、0.2、0.5、1 及 2%) 計算相對之亞磷酸重量，再分別稱取等重量的亞磷酸及氫氧化鉀藥品。配製溶液時，先將亞磷酸加入無菌水中，待完全溶解後再加入氫氧化鉀溶解，中和後之 NPA 的酸鹼值約為 pH 6.1–6.4，中和後之 NPA 於配製完成後於當日使用。

不同施用方法對馬鈴薯青枯病之溫室防治效果

本研究以馬鈴薯栽培品種「克尼伯」(*Solanum tuberosum* L. var. ‘Kennebec’) 參試，將已萌芽之馬鈴薯種薯切塊，每小塊至少留有 1 芽眼。切塊後置陰涼處 1 d，再種植於內含泥炭土栽培介質之 4 吋軟盆中生長，2 wk 後作

為試驗接種植株。利用低濃度之 NPA (0.05% 及 0.08%) 分別進行葉片噴施或土壤澆灌兩種處理，每 7 d 施用 1 次，連續 4 次。葉片噴施法利用 2 L 氣壓式噴水壺將植株所有葉片噴布 NPA，而土壤澆灌法則以 50 mL NPA 直接灌注土壤中，於第 1 次施用後 3 d 進行馬鈴薯青枯病菌接種。接種時將 50 mL 之青枯病菌懸浮液 (10^8 CFU mL^{-1}) 直接灌注土壤中，每組分別接種處理 5 株馬鈴薯，以未處理 NPA (0.00% NPA) 之植株作為對照組，試驗共進行 2 次。接種後的植株於溫室中定期觀察其發病情形及罹病等級，並計算罹病度 (disease severity)。青枯病罹病等級分為 6 級，0 級：無病徵；1 級：植物有逆境徵兆；2 級：1–33% 葉片萎凋或黃化；3 級：34–66% 葉片萎凋或黃化；4 級：67–100% 葉片萎凋或黃化；5 級：植株完全萎凋 (Swanson *et al*. 2007)，由下列公式計算其罹病度。

$$\text{Disease severity} = \frac{\Sigma ni \times i}{N \times 5} \times 100\%$$

其中，i：發病等級；ni：發病 i 級的株數；N：調查的總株數。

不同濃度亞磷酸施用對馬鈴薯青枯病病勢進展之影響

將不同濃度之 NPA (0.05、0.10、0.20、0.50、1.00 及 2.00%) 以土壤澆灌法進行處理，每株植物澆灌 50 mL NPA，每 7 d 施用 1 次，連續 4 次。第 1 次施用後 3 d 進行馬鈴薯青枯病菌接種，接種時將 50 mL 之青枯病菌懸浮液 (10^8 CFU mL^{-1}) 直接灌注土壤中，每組分別接種處理 6 株馬鈴薯，以未處理 NPA (0.00% NPA) 之植株作為對照組，試驗至少進行 2 次。接種後的植株，置於溫室中依上述青枯病罹病指數定期觀察其罹病等級及發病情形。

不同濃度亞磷酸對馬鈴薯生長之影響

分別將 50 mL 不同濃度之 NPA (0.05、0.10、0.20、0.50、1.00 及 2.00%) 利用土壤澆灌法處理種植 2 wk 後之植株，每 7 d 施用 1 次，連續 4 次。每組分別接種處理 6 株馬鈴薯，未處理 NPA (0.00% NPA) 之組別則以 50 mL 無菌水澆灌植株，試驗進行 2 次。處理後的植株於溫室中生長，5 wk 後測量植株之株高、鮮重 (fresh weight) 及薯塊等之生長情形。

亞磷酸對青枯病菌之抑制能力測定

本試驗採濾紙圓盤法及液體培養法，進行亞磷酸對青枯病菌之抑制能力評估，分述如下。

濾紙圓盤法

將直徑 0.8 cm 之濾紙圓盤 (Advantec Toyo Kaisha, Tokyo, Japan) 分別置於 0.1、0.5、1.0 及 2.0% 之 NPA 中，於室溫下浸漬 10 min 後將濾紙圓盤取出陰乾，再放置於已塗布有 0.1 mL 細菌懸浮液 (濃度約為 10^8 CFU mL^{-1}) 之營養洋菜培養基 (nutrient agar; NA) 平板上 (直徑 9 cm)。每一培養平板放 4 個濾紙圓盤，並以無菌水處理之濾紙圓盤作為對照組。將各處理之培養平板放置於 27℃無光照定溫箱內，培養 2 d 後，取出測量抑制圈大小 (扣除濾紙圓盤直徑 10 mm)。每處理 3 重複，試驗重複兩次。

液體培養法

事先以 0.22 μm 濾膜過濾 NPA，加入裝有 100 mL 之營養液體培養基 (nutrient broth; NB) (Difco Laboratories; Becton, Dickinson and Company, Franklin Lakes, NJ, USA) 的三角燒瓶中，並分別將其所含之 NPA 最終濃度調整為 0.1、0.5、1.0 及 2.0%。另以無菌水配製青枯病菌之細菌懸浮液，將濃度調整約為 10^8 CFU mL^{-1}，取 1 mL 之細菌懸浮液加入上述之 nutrient-NPA broth 三角燒瓶中，置於 25℃之震盪培養箱中 (轉速約 2× *g*)，每處理 2 重複。於培養 0、1、2 及 3 d 後取出，利用系列稀釋平板法計算菌落數後，推估菌株之濃度。

統計分析

試驗資料利用 SAS 9.1 版統計分析軟體先進行變方分析 (analysis of variance; ANOVA)，再以最小顯著性差異 (Fisher's least sig-

nificant difference; LSD) 測驗在 5% 顯著水準下，比較處理間平均值之差異。

結果

不同施用方法對馬鈴薯青枯病之溫室防治效果

本研究利用低濃度 NPA (0.05% 及 0.08%) 分別對馬鈴薯植株進行葉片噴施及土壤澆灌，每 7 d 施用 1 次，連續 4 次，於第 1 次施用後 3 d 進行馬鈴薯青枯病菌接種。於接種青枯病菌 21 d 後發現，以葉片噴施法使用 0.05% NPA 或 0.08% NPA 處理之馬鈴薯，其萎凋率 (wilt) 皆達 100%，而罹病度分別為 96% 及 92% (表 1)。同時，利用土壤澆灌法處理之馬鈴薯植株，使用 0.05% NPA 之植株萎凋率為 80%，罹病度為 68%；而使用濃度為 0.08% NPA 時，植株萎凋率為 60%，罹病度為 40% (表 1)；未處理 NPA (0.00% NPA) 僅接種青枯病菌之對照組植株，其萎凋率與罹病度皆達 100% (表 1)。由此可知，NPA 以土壤澆灌法使用對馬鈴薯青枯病之防治效果較以葉片噴施法更能降低青枯病發生之萎凋率及罹病度。

亞磷酸濃度對病勢進展之影響

利用土壤澆灌法分別將 6 種不同濃度 NPA 澆灌馬鈴薯植株，於接種 11 d 後發現，未處理 NPA 之對照組 (0.00% NPA) 與 0.05% NPA 澆灌之馬鈴薯植株已出現萎凋病徵，罹病度分別為 5% 及 11.67% (圖 1)。其他 5 種濃度 NPA (0.10、0.20、0.50、1.00 及 2.00%) 處理之組別，則未發現萎凋情形。接種 14 d 後，除了以 1.00% 及 2.00% NPA 澆灌之馬鈴薯植株未出現萎凋情形之外，其他 4 種 NPA 濃度處理組，皆可觀察到萎凋病徵出現。其中，罹病度大於 25% 的處理分別為 0.00% NPA (罹病度為 46.67%) 及 0.05% NPA 處理組 (罹病度為 28.35%)，而以 NPA 濃度 0.10、0.20 及 0.50% 澆灌之組別，罹病度皆低於 20%，分別為 13.33、16.67 及 8.33% (圖 1)。接種 18 d 後，0.00% NPA 罹病度為 66.67%，但 1.00% 及 2.00% NPA 澆灌之馬鈴薯植株仍未出現萎凋，其他 4 種 NPA 濃度處理組，以 0.05% NPA 澆灌之植株罹病度最高，達 50.00%；利用 NPA 濃度為 0.10、0.20 及 0.50% 澆灌者，罹病度皆低於 30.00%，分別為 25.00、23.33 及 21.67% (圖 1)。接種 22 d 後，0.0% NPA 罹病度為 73.33%，而 1.00% 及 2.00% NPA 澆灌之馬鈴薯植株仍未觀察到萎凋情形。但澆灌 0.05% NPA 之植株罹病度達 70.00%，NPA 濃度為 0.10、0.20 及 0.50% 澆灌者，罹病度皆低於 40.00%，分別為 36.67、36.67 及 28.33% (圖 1)。接種 28 d 後，0.00% NPA 罹病度已達 100.00%，澆灌 0.05% NPA 之植株罹病度為 83.33%，NPA 濃度為 0.10、0.20 及 0.50% 澆灌者，罹病度皆低於 50.00%，分別為 41.65、46.67 及 36.67%。然而，利用 1% 及 2% NPA 澆灌之馬鈴薯植株仍未觀察到萎凋情形 (圖 1)。綜上所述，濃度 0.10% 以上之 NPA 具有延遲青枯病發生的效果，能有效降低植株之罹

表 1. 亞磷酸-氫氧化鉀中和液不同施用方法對馬鈴薯青枯病發生之影響。

Table 1. Effects of neutralized phosphoric acid solution (NPA) by foliar spray and soil drench treatments on disease development of potato bacterial wilt.

	Foliar spray		Soil drench	
Treatment	Wilt (%)[z]	DS (%)[y]	Wilt (%)	DS (%)
NPA-0.05% + RS1118[x]	100[w]	96	80	68
NPA-0.08% + RS1118	100	92	60	40
NPA-0.00% + RS1118	100	100	100	100

[z] Wilt (%): percentage of completely wilted plants.
[y] DS (%): disease severity = [(Σnumber of diseased plants in each disease index × disease index)/(total number of plants inoculated × 5)] × 100%.
[x] RS1118: *Ralstonia solanacearum* strain 1118.
[w] Wilt and DS of potato was recorded 21 days post inoculation (dpi).

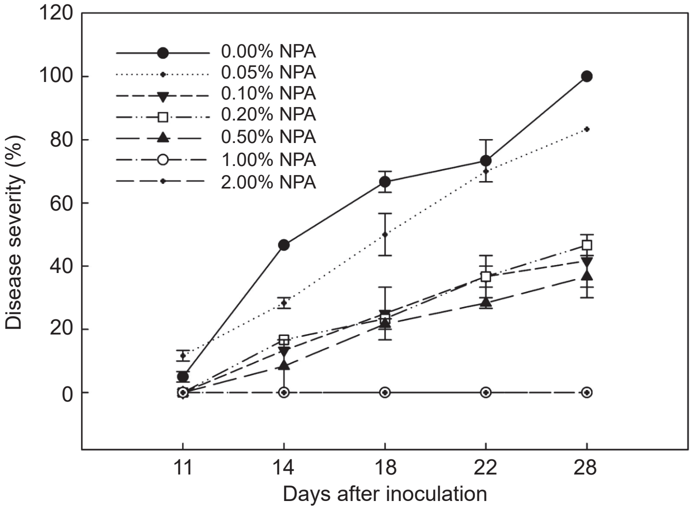

圖 1. 不同濃度之亞磷酸-氫氧化鉀中和液對馬鈴薯青枯病發生之影響。

Fig. 1. Effects of soil drench with different concentrations of neutralized phosphoric acid solution (NPA) on disease severity of potato bacterial wilt disease in greenhouse. Treatment means and standard errors were calculated from two independent experiments.

病度，其中以濃度 1.00% 及 2.00% NPA 效果最佳，於 28 d 的觀察期皆未有萎凋的病徵產生。

亞磷酸濃度對馬鈴薯生長之影響

選用 NPA 濃度為 0.10、0.50、1.00 及 2.00% 澆灌馬鈴薯植株，於處理後 5 wk 收取馬鈴薯之地上部測量其鮮重、株高及地下部薯塊生成情形。結果顯示，施用 2.00% NPA 會對植株之生長產生不良影響，出現抑制植株薯塊形成的現象。此外，施用 2.00% NPA 之植株鮮重平均為 9.96 g，與對照組的植株平均鮮重為 12.39 g 及施用 1.00% NPA 的植株平均鮮重 12.43 g 具有顯著之差異 (表 2)；株高方面，施用 2.00% NPA 對植株可產生輕微矮化的情形，其平均株高為 16.75 cm，與對照組雖無顯著之差異，但與施用其他濃度 NPA 之植株平均株高為 21.33–22.33 cm 相比，則有顯著性差異 (表 2)。

亞磷酸對青枯病菌抑制能力之測定

利用濾紙圓盤法進行培養基上之抑制能力試驗，結果發現以 0.1、0.5、1 及 2% NPA 處理之濾紙圓盤周圍皆無抑制圈之形成 (資料未顯示)，青枯病菌正常生長。由此可知，NPA 對青枯病菌無直接毒殺效果。

表 2. 不同濃度之亞磷酸-氫氧化鉀中和液對馬鈴薯生長之影響。

Table 2. Effects of soil drench with different concentrations of neutralized phosphoric acid solution (NPA) on growth of potato in greenhouse.

NPA treatment (%)	Fresh weight (g)/plant	Plant height (cm)	Tuber formation
0.0	12.39 a[z]	20.33 ab	Yes
0.1	10.78 ab	22.33 a	Yes
0.5	10.37 ab	21.33 a	Yes
1.0	12.43 a	22.17 a	Yes
2.0	9.96 b	16.75 b	No

[z] Means within the same column followed by the same letter are not significantly different at 5% by Fisher's least significant difference (LSD) test.

於液體培養法進行青枯病菌生長抑制能力試驗之結果顯示，將不同濃度之 NPA，加入營養液體培養基中，並未明顯影響培養基之酸鹼值，其 pH 值約為 7 左右。此外，NPA 可降低青枯病菌族群繁殖速度，但無法完全抑制，亦無直接毒殺青枯病菌的效果。與未加入 NPA 之對照組比較，隨著 NPA 濃度升高，菌株繁殖速度受到抑制的程度愈強，菌量濃度增加越慢 (圖 2)。其中，以 2.0% NPA 效果最為顯著，於 3 d 觀察期中，青枯病菌濃度皆維持與起始濃度相近，約為 6.4 Log CFU mL^{-1}，其次為 0.5% NPA 及 1.0% NPA。青枯病菌濃度於培養 1 d 後，菌量濃度增加約至 6.6–6.7 Log CFU mL^{-1}，2 d 後之菌量濃度即無明顯增加的現象；而加入 0.1% NPA，對青枯病菌族群繁殖速度亦有降低的效果，但其抑制效果不若其他 NPA 濃度強，菌株於培養 1 d 後菌量濃度增加約至 7.5 Log CFU mL^{-1}，之後呈緩慢增加的現象 (圖 2)。

討論

亞磷酸原本用途為植物緩效性磷肥的一種 (MacIntire *et al*. 1950)，自 1980 年代系統性殺菌劑「福賽得」(fosetyl-Al, aluminum tris-oethyl phosphonate) (Cohen & Coffey 1986) 被發現其代謝產物中的亞磷酸離子為主要抑制卵菌類病害之物質後，關於亞磷酸防治病害及其作用機制即引發廣泛的研究。目前亞磷酸已被開發成農藥及肥料，用於多種真菌病害之防治，尤其是疫病、露菌病等均有良好之防治成效 (Ann 2001)。於茄科作物病害防治方面，已證實利用地上部噴施法可減緩番茄及馬鈴薯疫病之病勢進展，並明顯降低其罹病度 (Tsai *et al*. 2009)。另外對番茄與番椒等作物之疫病，亦具防治效果 (Fenn & Coffey 1989)。近年來，已有研究報導指出亞磷酸對細菌病害亦有防治效果，如天竺葵、番茄之青枯病 (Norman *et al*. 2006; Lin & Wang 2011) 及番茄細菌性斑點病 (Wen *et al*. 2007; Wen *et al*. 2009)。本研究結果顯示，在溫室試驗條件下，利用土壤澆灌法施用亞磷酸，可有效防治馬鈴薯青枯病之發生及降低罹病度，濃度高時效果越顯著，若是利用葉面噴施法則其防治效果不佳。雖然亞磷酸於植物內的下移性良好 (Ann 2001)，但由於青枯病為土壤傳播性細菌，可於土壤中存活，並經由根部接觸或根部傷口等方式傳播。本研究採用土壤灌注法進行病原菌接種，以土

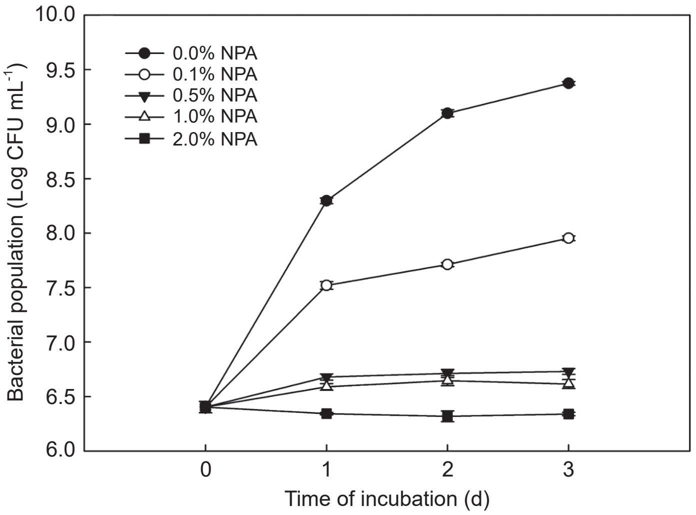

圖 2.　亞磷酸-氫氧化鉀中和液對馬鈴薯青枯病菌生長之影響。

Fig. 2.　Effect of different concentrations of neutralized phosphoric acid solution (NPA) on growth of *Ralstonia solanacearum* strain 1118 (RS1118) in nutrient broth culture. Treatment means and standard errors were calculated from two independent experiments.

壤澆灌法施用時可直接且快速地產生作用，因此較葉面噴施法使用亞磷酸的效果明顯。此外，Norman *et al*. (2006) 及 Lin & Wang (2011) 之研究結果亦顯示，利用土壤澆灌法每株施用 50 mL 之亞磷酸可有效防治天竺葵及番茄青枯病的發生。

關於亞磷酸防治真菌病害之作用機制，目前已被證實者包括直接抑制病原菌之菌絲或孢子發芽以達到保護寄主的功效，與間接誘導植物增強抗病性 (Grant *et al*. 1990; Guest & Bompeix 1990)，但全程詳細的機制尚未完全明瞭。本研究經由生體外試驗結果顯示，亞磷酸可抑制青枯病菌之繁殖速度，且隨著亞磷酸濃度增加，抑制效果越顯著，但無法完全抑制其繁殖亦無法直接殺死病原細菌 (圖 2)。此結果或許可說明於溫室試驗中，澆灌濃度 0.1% 以上之 NPA 具有明顯延遲青枯病發生並減緩病勢進展的原因 (圖 1)。然而，一旦植株受到病原菌感染後，即便使用亞磷酸，植株仍會逐漸萎凋、死亡。前人研究亦發現相似的結果，生體外利用 0.50% 及 0.75% 亞磷酸，可降低番茄細菌性斑點病菌 (*Xanthomonas perforans*) 的繁殖速度，且 0.50% 及 0.75% 亞磷酸之抑制效果相當；於溫室試驗中，利用葉面噴施法使用亞磷酸亦能降低病勢之進展，但未觀察到亞磷酸具有誘導植株增強抗性之效果 (Wen *et al*. 2009)。此外，Norman *et al*. (2006) 之研究結果亦顯示 1.00% 亞磷酸可抑制青枯病菌的生長，且連續澆灌 2 次或 4 次之亞磷酸至盆缽內的土壤中，能有效保護天竺葵不受感染。於本研究中亦顯示亞磷酸具有類似抑菌劑之作用，藉此達到防治病害之效果，但仍需進一步闡明相關的作用機制。

亞磷酸之酸鹼值低，須與強鹼物質進行中和，使酸鹼值為 pH 5.5–6.5 後施用才能避免對植物造成藥害 (Ann 2001)。行政院農業委員會農業試驗所已研發出一種簡便的亞磷酸溶液配製法 (Ann *et al*. 2000)，將適量之亞磷酸與中和劑氫氧化鉀以 1：1 (w/w) 等量秤重，接著將亞磷酸先行溶解於水中後，再加入氫氧化鉀溶解，中和後之 NPA 酸鹼值即為弱酸性 (pH 6.0–6.8)，可安全使用，對植物不會產生藥害。除了亞磷酸溶液之酸鹼值可對植物生長造成影響之外，因不同植物對亞磷酸的感受性不同，一旦使用濃度超出植物所能承受之範圍，亦會對植物造成傷害。相關研究顯示，連續每周澆灌 5 次 2% 亞磷酸，會造成天竺葵植株葉片黃化與其乾重 (dry weight) 減輕之影響，而使用 1.00% 亞磷酸，則無上述的不良作用 (Norman *et al*. 2006)。本研究結果發現，施用高濃度 2.00% NPA 可有效防治青枯病發生，但是卻導致馬鈴薯植株出現輕微矮化的現象，同時影響植株地下部薯塊的生成，降低生產能力；而 1.00% NPA 則不會對植株薯塊生成能力造成影響，且能提供良好之防治效果。因此，使用之 NPA 濃度建議不宜超過 1.00%，才能避免馬鈴薯產生藥害。亞磷酸為磷肥的一種，有助於植物的生長 (Rickard 2000)，但於本研究中，使用 0.10–1.00% NPA 對馬鈴薯之植株高度及其鮮重未有顯著之增加，可能與馬鈴薯種植於盆缽中 (4 吋黑色軟盆)，生長受到限制有關。未來將進一步進行田間測試，以瞭解 NPA 對馬鈴薯生長之影響。

馬鈴薯青枯病的防治首重預防，田間一旦發病後，病勢進展非常快速，防治十分困難。本研究發現利用土壤澆灌法施用 0.10–1.00% NPA 可有效防治青枯病之發生，降低罹病度。由於青枯病菌可藉由傷口、受污染的水源、種苗或病土傳播，若能配合健康種薯及注意田間衛生等方法，方能發揮最佳防治效果。以上資訊，可提供相關農政單位擬定馬鈴薯青枯病防治策略之參考。此外，亞磷酸為非化學合成農藥，研究指出亞磷酸鈉於 50,000 mg L^{-1} 高劑量下，對老鼠無致癌性 (Quest *et al*. 1991)，對人畜無毒，採收的農作物沒有農藥殘留的問題，亦不會造成環境汙染及衝擊生態環境。而且亞磷酸價格便宜、使用方式簡便，對農民助益良多。

誌謝

本研究工作承本研究室同仁林江美華小姐及陳幸葵小姐協助試驗進行與倪蕙芳主任及黃哲倫助理研究員於文稿之指正，特此誌謝。

引用文獻

Ann, P. J. 2001. Control of plant diseases with non-pesticide compound-phosphorous acid. Plant Pathol. Bull. 10:147–154. (in Chinese with English abstract)

Ann, P. J., T. F. Hsieh, J. N. Tsai, I. T. Wang, and C. Y. Lin. 2000. A simple method for use of phosphorous acid and spectra of disease control. Plant Pathol. Bull. 9:179. (abstract in Chinese)

Chiou, Y. S. 2002. Characteristics of strains of *Ralstonia solanacearum* recently affecting potatoes in central Taiwan. Master Thesis, Department of Plant Pathology, National Chung Hsing University. Taichung, Taiwan. 46 pp. (in Chinese with English abstract)

Chuang, M. F., S. F. Lo, and C. Y. Lin. 2015. Effect of temperature on virulence of *Ralstonia solanacearum* biovars and response of potato cultivars (lines) to bacterial wilt. J. Taiwan Agric. Res. 64:89–98. (in Chinese with English abstract)

Ciampi, L. and L. Sequeira. 1980. Influence of temperature on virulence of race 3 strains of *Pseudomonas solanacearum*. Amer. Potato J. 57:307–317.

Cohen, Y. and M. D. Coffey. 1986. Systemic fungicides and the control of oomycetes. Annu. Rev. Phytopathol. 24:311–338.

Denny, T. P. 2006. Plant pathogenic *Ralstonia* species. p.573–644. *in*: Plant-Associated Bacteria. (Gnanamanickam, S. S., ed.) Springer. Dordrecht, The Netherlands. 712 pp.

Elphinstone, J. G. 2005. The current bacterial wilt situation: A global overview. p.9–28. *in*: Bacterial Wilt Disease and the *Ralstonia solanacearum* Species Complex. (Allen, C., P. Prior, and A. C. Hayward, eds.) APS Press. St. Paul, MN. 510 pp.

Fegan, M. and P. Prior. 2005. How complex is the *Ralstonia solanacearum* species complex? p.449–461. *in*: Bacterial Wilt Disease and the *Ralstonia solanacearum* Species Complex. (Allen, C., P. Prior, and A. C. Hayward, eds.) APS Press. St. Paul, MN. 510 pp.

Fenn, M. E. and M. D. Coffey. 1989. Quantification of phosphonate and ethyl phosphonate in tobacco and tomato tissues and significance for the mode of action of two phosphonate fungicides. Phytopathology 79:76–82.

Genin, S. and C. Boucher. 2004. Lessons learned from the genome analysis of *Ralstonia solanacearum*. Annu. Rev. Phytopathol. 42:107–134.

Grant, B. R., R. H. Dunstan, J. M. Griffith, J. O. Niere, and R. H. Smillie. 1990. The mechanism of phosphonic (phosphorous) acid action in *Phytophthora*. Aust. Plant Pathol. 19:115–121.

Guest, D. and G. Bompeix. 1990. The complex mode of action of phosphonates. Aust. Plant Pathol. 19:113–115.

Hayward, A. C. 1991. Biology and epidemiology of bacterial wilt caused by *Pseudomonas solanacearum*. Annu. Rev. Phytopathol. 29:65–87.

Hayward, A. C. 2000. *Ralstonia solanacearum*. p.32–42. *in*: Encyclopedia of Microbiology. Vol. 4. (Lederberg, J., ed.) Academic Press. San Diego, CA. 1142 pp.

Hsu, S. T. 1991. Ecology and control of *Pseudomonas solanacearum* in Taiwan. Plant Prot. Bull. 33:72–79. (in Chinese with English abstract)

Johnson, D. A., D. A. Inglis, and J. S. Miller. 2004. Control of potato tuber rots caused by oomycetes with foliar applications of phosphorous acid. Plant Dis. 88:1153–1159.

Kelman, A. 1954. The relationship of pathogenicity in *Pseudomonas solanacearum* to colony appearance on tetrazolium medium. Phytopathology 44:693–695.

Lin, C. H. 2008. Application of Population Profiling and Detection of *Ralstonia solanacearum* on Integrated Management of Tomato Bacterial Wilt. Ph.D. Dissertation, Department of Plant Pathology, National Chung Hsing University. Taichung, Taiwan. 101 pp.

Lin, C. H. and J. F. Wang. 2011. Phosphorous acid salt: A promising chemical to control tomato bacterial wilt. SP-IPM Technical Innovation Brief 13. http://www.spipm.cgiar.org/c/document_library/get_file?p_l_id=17830&folderId=18484&name=DLFE-3821.pdf (visit on 2/12/2018)

MacIntire, W. H., S. H. Winterberg, L. J. Hardin, A. J. Sterges, and L. B. Clements. 1950. Fertilizer evaluation of certain phosphorus, phosphorous, and phosphoric materials by means of pot cultures. Agron. J. 42:543–549.

Norman, D. J., J. Chen, J. M. F. Yuen, A. Mangravita-Novo, D. Byrne, and L. Walsh. 2006. Control of bacterial wilt of geranium with phosphorous acid. Plant Dis. 90:798–802.

Quest, J. A., K. L. Hamernik, R. Engler, W. L. Burnam, and P. A. Fenner-Crisp. 1991. Evaluation of the carcinogenic potential of pesticides. 3. Aliette. Regul. Toxicol. Pharmacol. 14:3–11.

Rickard, D. A. 2000. Review of phosphorus acid and its salts as fertilizer materials. J. Plant Nutr. 23:161–180.

Swanson, J. K., L. Montes, M. Mejia, and C. Allen. 2007. Detection of latent infections of *Ralstonia solanacearum* race 3 biovar 2 in geranium. Plant Dis.

91:828–834.

Tsai, J. N., P. J. Ann, I. T. Wang, S. Y. Wang, and C. Y. Hu. 2009. Control of phytophthora late blight of potato and tomato with neutralized phosphorous acid. J. Taiwan Agric. Res. 58:185–195. (in Chinese with English abstract)

Wen, A., B. Balogh, M. T. Momol, J. B. Jones, S. M. Olson, L. S. Ritchie, P. D. Roberts, R. Systma, and C. W. Meister. 2007. Management of bacterial spot of tomato with phosphorous acid containing compounds. Phytopathology 97:S121.

Wen, A., B. Balogh, M. T. Momol, S. M. Olson, and J. B. Jones. 2009. Management of bacterial spot of tomato with phosphorous acid salts. Crop Prot. 28:859–863.

Wicker, E., L. Grassart, R. Coranson-Beaudu, D. Mian, C. Guilbaud, M. Fegan, and P. Prior. 2007. *Ralstonia solanacearum* strains from Martinique (French West Indies) exhibiting a new pathogenic potential. Appl. Environ. Microbiol. 73:6790–6801.

Wilson, E. E., F. M. Zeitoun, and D. L. Fredrickson. 1967. Bacterial phloem canker, a new disease of Persian walnut trees. Phytopathology 57:618–621.

Wu, Y. F., C. H. Lin, T. M. Chen, Y. F. Huang, S. H. Chen, J. H. Wang, and A. S. Cheng. 2010. Ecological survey of brown rot potato in Dounan, Yunlin. Plant Pathol. Bull. 19:87. (in Chinese with English abstract)

Wu, Y. F., C. H. Lin, J. F. Wang, and A. S. Cheng. 2011. Population density of *Ralstonia solanacearum* potato strain, phylotype II/race 3/biovar 2, and incidence of potato bacterial wilt in fields Dounan, Yunlin County. Plant Pathol. Bull. 20:68–77. (in Chinese with English abstract)

Control of Bacterial Wilt of Potato with Neutralized Phosphorous Acid

Ching-Yi Lin[1,*] and Hui-Ju Lin[2]

Abstract

Lin, C. Y. and H. J. Lin. 2018. Control of bacterial wilt of potato with neutralized phosphorous acid. J. Taiwan Agric. Res. 67(4):377–386.

Ralstonia solanacearum is the causal agent of the plant disease known as bacterial wilt, which is a serious threat to potato industry worldwide. Control of bacterial wilt is very difficult, largely being dependent on good crop management practices such as the uses of healthy tubers, pathogen-free soil and crop rotation. A greenhouse study was conducted to determine effects of neutralized phosphoric acid solution (NPA) on control of bacterial wilt of potato. The disease severity of inoculated potato plants was decreased to 40% when 0.08% NPA applied as soil drench, whereas the disease severity of inoculated potato plants was 92% by foliar spray application of 0.08% NPA. The results indicated that soil drench application of NPA provided better disease control than the foliar spray. Concentration effects of NPA on control of bacterial wilt of potato were evaluated through soil drench. A significant reduction in disease severity was observed at 0.10% and 2.00% NPA 28 days after inoculation and the disease severity ranging from 0.00% to 46.67%. At low concentration of 0.05% NPA, disease severity slightly decreased to 83.33%. However, treatment with a high concentration of 2.00% NPA was found to stunt potato growth and inhibit tuberization in greenhouse. Thus, application of NPA concentration more than 1.00% is not feasible. The mechanism of action of NPA is still unclear, as it only affects multiplication of *R. solanacearum in vitro*.

Key words: Phosphoric acid, Potato, Bacterial wilt.

Received: April 17, 2018; Accepted: June 4, 2018.

[*] Corresponding author, e-mail: eris2024@dns.caes.gov.tw

[1] Assistant Research Fellow, Department of Plant Protection, Chiayi Agricultural Experiment Branch, Taiwan Agricultural Research Institute, Chiayi, Taiwan, ROC.

[2] Research Assistant, Department of Plant Protection, Chiayi Agricultural Experiment Branch, Taiwan Agricultural Research Institute, Chiayi, Taiwan, ROC.

台灣農業研究 (J. Taiwan Agric. Res.) 67(4):387–400 (2018)
DOI:10.6156/JTAR.201812_67(4).0007

研究報告

柑橘褐斑病之病原特性及防治藥劑篩選

黃巧雯[1] 吳昭蓉[2] 楊宏仁[3] 賴素玉[4] 倪蕙芳[5,*]

摘要

黃巧雯、吳昭蓉、楊宏仁、賴素玉、倪蕙芳。2018。柑橘褐斑病之病原特性及防治藥劑篩選。台灣農業研究 67(4):387–400。

柑橘褐斑病在 2012–2013 年間於台灣之「茂谷柑」、「佛利蒙柑」及「帝王柑」等柑橘園區之春梢期陸續發生。本研究除針對該病原菌進行生理特性調查外，亦對不同柑橘品種之病原性及其防治藥劑進行篩選評估。柑橘褐斑病菌分離株 (*Alternaria alternata* Alt-001) 可在「茂谷柑」、「明尼橘柚」、「桶柑」、「佛利蒙柑」、「晚崙西亞」、「豔陽柑」及「台農天王柑」等柑橘品種之離葉嫩葉傷口接種時，造成褐色病斑，但在粗皮檸檬之嫩葉上則不造成病斑。本病原最適菌絲生長溫度為 25℃，而 25–30℃為孢子發芽最適溫度。測試殺菌劑對其菌絲生長及孢子發芽影響時，結果顯示快得寧在 10 mg a.i. L^{-1} 有效濃度下可有效抑制菌絲生長，抑制率達 99.2%；而快得寧、氫氧化銅、鋅錳乃浦及扶吉胺等 4 種藥劑可顯著降低病原菌之孢子發芽，其中植株噴布快得寧、氫氧化銅及鋅錳乃浦藥劑處理 21 d 後，*A. alternata* Alt-001分離株在其葉片上之孢子發芽率仍顯著低於噴布水之對照組。快得寧、氫氧化銅、鋅錳乃浦及扶吉胺為目前官方推薦柑橘真菌性病害之防治藥劑，從藥效評估結果，上述藥劑可一併作為防治柑橘褐斑病之用藥。

關鍵詞：柑橘、柑橘褐斑病、柑橘褐斑病菌、藥劑篩選。

前言

柑橘 (*Citrus reticulata*) 為芸香科 (Rutaceae) 柑橘屬 (*Citrus* L.) 多年生果樹，原產於中國，種類繁多、型態特性各異，可適應不同地區的氣候，為台灣重要果樹產業之一。目前栽培品種極多，包括「椪柑」、「柳橙」、「桶柑」、「文旦柚」、「白柚」、「檸檬」、「葡萄柚」、「金柑」、「茂谷柑」、「萊姆」、「明尼桔柚」、「晚崙西亞橙」、「臍橙」等。依農業委員會農業統計年報資料 (http://agrstat.coa.gov.tw/sdweb/public/official/OfficialInformation.aspx) 顯示，2016 年全台柑橘種植面積為 26,210 ha，年產量為 462,638 Mg，其中以「椪柑」之栽培面積最大為 5,572 ha，其次為「柳橙」5,244 ha，「文旦柚」4,286 ha，「桶柑」3,175 ha。柑橘產區分布全台，其中「椪柑」主要栽培地為嘉義縣、台中市、台南市及苗栗縣；「柳橙」則主要分布於台南市、雲林、嘉義縣及南投縣；「桶柑」分布於新竹縣、苗栗縣、台中市；「文旦」則分布於台南市、花蓮縣及苗栗縣；「檸檬」大多種植於屏東縣；「海梨柑」主要產區為新竹縣；「金柑」主要產區為宜蘭縣；「白柚」主要種植於台南市及台東縣。

台灣柑橘病害有由 *Citrus tristeza virus* (CTV) 引起的萎縮病；由 *Citrus tatter leaf virus* (CTLV) 引起的破葉病；由 *Citrus exocortis viroid* (CEVd) 引起的鱗砧病；由 *Candidatus*

投稿日期：2018 年 4 月 20 日；接受日期：2018 年 6 月 6 日。
* 通訊作者：hfni@dns.caes.gov.tw
1 農委會農業試驗所植物病理組助理研究員。台灣 台中市。
2 農委會農業試驗所嘉義農業試驗分所植物保護系助理研究員。台灣 嘉義市。
3 農委會農業試驗所嘉義農業試驗分所研究員兼分所長。台灣 嘉義市。
4 農委會農業試驗所嘉義農業試驗分所植物保護系研究助理。台灣 嘉義市。
5 農委會農業試驗所嘉義農業試驗分所植物保護系副研究員兼系主任。台灣 嘉義市。

Liberibacter asiaticus 引起的黃龍病；由 *Xanthomonas axonopodis* pv. *citri* 引起的潰瘍病；由 *Phytophthora parasitica*、*Phytophthora palmivora*、*Phytophthora citrothora*、*Phytophthora cinnamomi* 及 *Phytophthora citricola* 等疫病菌引起的裾腐病、根腐病、金柑芽葉疫病及果實褐腐病；由 *Guignardia citricarpa* 引起的黑星病；由 *Diaporthe citri* 引起的黑點病；由 *Elsinoe fawcettii* 引起的瘡痂病；由 *Penicillium digitatum* 及 *Penicillium italicum* 引起的綠黴病與青黴病；由 *Botryosphaeria rhodina* 及 *Diapothe citri* 引起的蒂腐病；由 *Colletotrichum gloeosporioides* 引起的炭疽病；由 *Erythricium salmonicolor* 引起的赤衣病；由 *Mycosphaerella citri* 引起的油斑病；由 *Oidium tingitaninum* 引起的白粉病；另外，亦有由根腐線蟲 (*Pratylenchus coffeae*) 與柑橘線蟲 (*Tylenchulus semipenetrans*) 引起之線蟲病害 (Hsu *et al*. 2002; Tsay *et al*. 2002)。而在 2012 年 4 月中旬分別於台中市東勢區「茂谷柑」(‘Murcott tangor’) 及南投縣中寮鄉「帝王柑」(‘Gonggan’) 與「佛利蒙柑」(‘Fremont’) 之新梢褐化病害樣品上，經病原分離、鏡檢病原形態及依柯霍氏法則 (Koch’s postulates) 進行病原性測定後，確認為由 *Alternaria alternata* 引起之柑橘褐斑病 (Alternaria brown spot; ABS) (Ni *et al*. 2015)，為台灣新紀錄病害；且在 2016 及 2017 年之 4 月中下旬，陸續在台中市東勢區不同農友種植之「茂谷柑」春梢上發生本病害，造成嚴重新梢落葉。

柑橘褐斑病最早於 1903 年在澳洲之柑橘 ‘Emperor mandarin’ 品種上發生 (Cobb 1903)，之後在美國佛羅里達之「紅橘」(‘Dancy tangerines’) (Whiteside 1976)，以色列、南非、西班牙、祕魯及希臘等國之「明尼桔柚」(‘Minneola tangelo’) (Solel 1991; Swart *et al*. 1998; Vicent *et al*. 2000; Elena 2006; Marín *et al*. 2006)，以及阿根廷與巴西之「茂谷柑」品種上陸續發現本病害 (Peres *et al*. 2003)。本病害在嫩葉、枝條及果實上造成黑色壞疽病斑，嚴重時導致落葉、落果及枝條枯萎死亡 (Reis *et al*. 2006)，進而影響樹勢生長、減少果實產量及降低果實商品價值 (Timmer *et al*. 2000b)。

目前國外在柑橘褐斑病的防治策略上，已知可透過避免作物過度密植、避免施用過量氮肥及避免過度灌溉等栽培管理方法降低病害發生，而防治藥劑部分，已知施用依普同 (iprodione) 及銅劑 (copper products) 等可有效地控制柑橘褐斑病發生 (Solel *et al*. 1997; Timmer *et al*. 2000b)。惟在國內雖已知 *A. alternata* 為引起柑橘褐斑病之病原菌 (Ni *et al*. 2015)，卻無正式推薦藥劑供農友防治，因此本研究除對柑橘褐斑病菌生理特性及病原性進行瞭解外。另外，就現已推薦防治柑橘其他真菌性病害之藥劑，進行本病害之室內防治藥劑篩選，期藉此能篩選出有效藥劑進行柑橘褐斑病之防治，提供農友防治資訊。

材料與方法

病原菌分離與保存

將田間罹患褐斑病之柑橘葉片病斑攜回實驗室，以解剖顯微鏡 (SMZ1500, Nikon, Tokyo, Japan) 觀察，若發現病斑上有黑褐色孢子及菌絲等病兆者，則直接挑取病原置於以乳酸 (lactic acid) 酸化馬鈴薯葡萄糖瓊脂 (potato dextrose agar; PDA) 之平板上。此酸化馬鈴薯葡萄糖瓊脂 (acidified PDA; APDA) 酸鹼值 3.8，配製方法為以 750 μL 50% (v/v) 乳酸溶液加入 300 mL PDA (Merck KGaA, Darmstadt, Germany)。若無病兆者，則直接將罹病之病斑組織，以 0.5% 次氯酸鈉漂洗消毒 30 s，繼而以 70% 酒精消毒 30 s，最後再以無菌水漂洗 2 次。自然風乾後，利用滅菌過之解剖刀切取病健部相鄰組織，置於 APDA 培養基上分離。其分離所得之菌株置於 PDA 中純化後，待其產孢再進行單孢分離，並將分離株保存於 PDA 斜面培養基及無菌水中，置於 10℃備用。本研究使用之 Alt-001 及 Alt-008 菌株，分別由南投縣中寮鄉「佛利蒙柑」及台中市東勢區「茂谷柑」之罹病葉片所分離之 *A. alternata* 菌株。

病原菌型態及分子鑑定

將 Alt-001 及 Alt-008 菌株培養於 PDA 培養基上，置於 25℃培養約 7 d 後觀察菌落生長

型態。待其產孢後，以滅菌之針頭挑取培養基上之分生孢子置於載玻片上，並以干涉位相差顯微鏡 (differential interference contrast; DIC; Nikon 80i, Nikon, Tokyo, Japan) 進行孢子型態觀察，再以 NIS-Elements BR 3.0 軟體 (Nikon, Tokyo, Japan) 測量 50 個以上分生孢子之長度及寬度。分子鑑定方式，則是刮取菌絲置於微量離心管中，加入 0.5 N NaOH 溶液，將菌絲磨碎後，以 13,800× *g* 離心 (Heraeus Fresco 21 Microcentrifuge, Thermo Fisher Scientific, Waltham, MA, USA) 5 min。吸取上清液，與 9 倍體積之 0.1 M Tris buffer (pH 8.0) 混合，直接吸取混合後之溶液，以 Taq DNA Poly (Protech) 聚合酶酵素進行 DNA 聚合酶鏈鎖反應，增幅 endopolygalacturanase (*endo*PG) 片段時所使用之引子對為 5'-TGTGCTACCATGGTTCTTTCC-3' 及 5'-TGTAAACCTTAGCGCCATCA-3' (Peever *et al*. 2005)；增幅 internal transcribed spacer (ITS) 片段時所使用之引子對為 ITS1 及 ITS4 (White *et al*. 1990)，增幅後產物委由源資國際生物科技股份有限公司 (Tri-I Biotech Inc.) 進行定序。親緣樹狀圖建構方式參照 Garganese *et al*. (2016) 並略加修改，除 Alt-001 及 Alt-008 兩菌株序列為自行增幅外，演化樹中其他菌株的序列資料由 National Center for Biotechnology Information (NCBI) Genbank (https://www.ncbi.nlm.nih.gov/genbank/) 網站依其不同菌株編號查詢後獲得。將同一菌株的 *endo*PG 及 ITS 片段頭尾相連後，所有菌株序列使用 MEGA7 軟體中的 ClustalW 功能按預設參數進行並列排序 (alignment)，並以 Maximum Likelihood 演算法及 Tamura-Nei model 進行類緣關係分析，建構演化樹，bootstrap 重複數皆設定為 1,000。

柑橘褐斑病分離株對不同柑橘品種之病原性測定

供試柑橘品種計有「晚崙西亞」、「佛利蒙柑」、「桶柑」、「明尼橘柚」、「茂谷柑」、「豔陽柑」、「台農天王柑」及「粗皮檸檬」等 8 種不同柑橘品種，其中「茂谷柑」和「明尼橘柚」兩品種除進行離葉接種外，亦進行植株上之接種試驗。

植株上接種方式如下：將 Alt-001 分離株培養於 PDA 7 d 後，以無菌水洗下培養皿內之分生孢子，並利用血球計數器 (hemacytometer, Bright-Line, Miami, FL, USA) 計算孢子濃度，再以無菌水製成孢子懸浮液 (10^5 spores mL^{-1})。續將孢子懸浮液均勻噴布於植株嫩葉上，接種後之嫩葉隨即進行套袋保濕 2 d 後去除塑膠袋，接種後每週調查病勢進展情形，對照組則以無菌水進行接種。

另外，離葉接種方式如下：將不同柑橘品種健康嫩葉由植株取下後，以滅過菌 3 號蟲針在嫩葉葉背上製造傷口。將上述以無菌水配製孢子懸浮液 (10^5 spores mL^{-1})，取 20 μL 滴於傷口上，每片葉片於左右兩側各處理一點，每種柑橘品種處理 5 片離葉，並以無菌水作為對照組。再將接種後葉片保濕於密封塑膠盒中，分別放置於 25℃黑暗之定溫箱中，逐日觀察病斑進展情形，本試驗重複進行 2 次。

溫度對病原菌菌絲生長及孢子發芽之影響

將 Alt-001 及 Alt-008 菌株分別移植於 PDA 培養基，置於室溫培養 7 d 後，以直徑 0.5 cm 滅菌過之打孔器切取菌絲邊緣，置於 PDA 平板中央。分別置於 10、15、20、25、30、35 及 40℃之定溫箱中黑暗培養，於培養後第 9 天測量其菌絲生長之直徑，每個處理 5 重複，本試驗重複進行 2 次。另外，將 Alt-001 及 Alt-008 菌株分別培養於 PDA，培養約 7 d 待其產孢後，以無菌水配製約 200 spores/50 μL 之孢子懸浮液，置於 3 凹載玻片上，並將玻片保濕於塑膠培養皿中，分別置於 10、15、20、25、30、35 及 40℃之定溫箱中，6 h 後取出於顯微鏡計算孢子之發芽情形，每個處理 6 重複，本試驗重複進行 2 次。

化學藥劑對 *A. alternata* 分離株菌絲生長之影響

將供試菌株 Alt-001 及 Alt-008 分別移植至 PDA 培養基，室溫培養 7 d 後，以滅菌過之打孔器 (孔徑 0.5 cm) 切取菌絲塊供試，並利用藥劑平板測試法測定供試藥劑之抑菌效果。所測試的藥劑為目前官方推薦在柑橘真菌性病

害防治之藥劑，藥劑種類如下：15.0% 易胺座可濕性粉劑 (wettable powders; WP) (imibenconazole，大勝化學工業股份有限公司，台灣台北市)、40.0% 快得寧 WP (oxine-copper，嘉泰企業股份有限公司，台灣桃園市)、70.0% 甲基多保淨 WP (thiophanate methyl，瑞繐股份有限公司，台灣台北市)、72.0% 波爾多 WP (bordeaux mixture，台灣龍燈股份有限公司，台灣台北市)、80.0% 鋅錳乃浦 WP (mancozeb，安農股份有限公司，台灣新北市)、81.3% 嘉賜銅 WP (kasugamycin + copper oxychloride，大勝化學工業股份有限公司，台灣台北市)、25.0% 亞托敏水懸劑 (suspension concentrates; SC) (azoxystrobin，台灣先正達股份有限公司，台灣台北市)、39.5% 扶吉胺 SC (fluazinam，台灣石原產業股份有限公司，台灣台北市)、77.0% 氫氧化銅 WP [copper(II) hydroxide，瑞芳植物保護股份有限公司，台灣嘉義縣]、27.1% 三元硫酸銅 SC (tribasic copper sulfate，日星實業股份有限公司，台灣台北市) 等 10 種藥劑，配置成含有效成分濃度為 1 mg a.i. L^{-1}、10 mg a.i. L^{-1}、100 mg a.i. L^{-1} 之 PDA 培養基。另以不添加藥劑之 PDA 培養基作為對照，再將直徑 0.5 cm 的菌絲塊，菌絲面朝下置入直徑 9 cm 之含藥的 PDA 培養基平板中央，置於 25℃之定溫箱中黑暗培養。培養後第 8 天，測量其菌絲生長之直徑，每個處理 5 重複，本試驗重複進行 3 次。試驗結果按下列公式換算藥劑對菌絲之生長抑制率：抑制率 (%) = {[對照組生長直徑 (cm) − 藥劑處理組生長直徑 (cm)]/ 對照組生長直徑 (cm)} × 100%。

化學藥劑對 *A. alternata* 分離株孢子發芽之影響

以滅菌微量吸管吸取 30 μL 上述不同藥劑之溶液，置於 3 凹載玻片之凹槽內，再以無菌微量吸管吸取 2 μL 供試之 Alt-001 及 Alt-008 菌株孢子懸浮液 (約 200 spores μL^{-1})，滴於含農藥 (嘉賜銅、鋅錳乃浦、氫氧化銅、波爾多、甲基多保淨、快得寧及扶吉胺等 7 種藥劑) 之載玻片凹槽內，並混合均勻，使凹槽內混合液之藥劑有效成分濃度為分別為 1 mg a.i. L^{-1}、10 mg a.i. L^{-1} 及 100 mg a.i. L^{-1}。供試載玻片並置於加有 1–5 mL 無菌水之 9 cm 塑膠培養皿中保濕，將培養皿置於 25℃之定溫箱中經 6 h 後，於顯微鏡下逢機計算 100 個孢子之發芽率，每個處理 2 皿，6 重複，本試驗重複進行 2 次。以滅菌逆滲透水處理作為對照組。

化學藥劑噴施於柑橘葉片不同時間後對 *A. alternata* Alt-001 菌株孢子發芽之影響

本試驗使用之植物材料為盆植於溫室之「茂谷柑」。所使用之藥劑濃度均按植物保護手冊推薦，分別為 40.0% 快得寧可濕性粉劑稀釋 500×、72.0% 波爾多可濕性粉劑稀釋 500×、70.0% 甲基多保淨可濕性粉劑稀釋 1,000×、80.0% 鋅錳乃浦可濕性粉劑稀釋 500×、77.0% 氫氧化銅可濕性粉劑稀釋 800× 及 39.5% 扶吉胺水懸劑稀釋 2,000× 等 6 種藥劑。將上述藥劑分別噴布於「茂谷柑」植株嫩葉上，於噴藥後 3、5、7、14 及 21 d 分別採集葉片，並將已配製好 Alt-001 菌株之孢子懸浮液 (約 200 spores 20 μL^{-1})，以無菌微量吸管吸取 20 μL 之孢子懸浮液滴於含藥劑之葉面上左右兩側，每處理 8 重複。置於室溫下 6 h 後，取出並以 0.05% trypan blue 進行孢子染色，於顯微鏡下計算每 100 個孢子之發芽率。植株噴布水之葉片為對照組，本試驗重複進行 2 次。

統計分析

各項處理之試驗資料利用 SAS Enterprise Guide 7.1 版統計分析先進行變方分析 (analysis of variance; ANOVA)，再以最小顯著性差異 (least significant difference; LSD) 測驗，在 5% 顯著水準下比較處理間平均值之差異。

結果

柑橘褐斑病之病徵

本研究於栽植「佛利蒙柑」、「茂谷柑」及「帝王柑」品種之柑橘園區陸續調查到褐斑病 (alternaria brown spot) 的發生，柑橘褐斑病主要危害嫩葉。罹病時，發病初期葉片呈現褐色至深褐色水浸狀小斑點，病斑逐漸擴大成圓形、不整圓形或輪紋狀褐色病斑，有時病斑外圍會沿著葉脈呈細長條狀網紋斑，病斑周圍具

有明顯的黃色暈環 (圖 1A–1C)。有時在罹病葉片上可觀察到病兆 (圖 1D)，於解剖顯微鏡下其病兆範圍內可明顯觀察到黑色分生孢子鏈產生，葉片被害嚴重時會導致大量落葉。

柑橘褐斑病菌之培養形態與分子鑑定

柑橘褐斑病菌培養於 PDA 平板於 25℃黑暗培養 8 d 後，菌落為深墨綠色、菌絲茂密生長 (圖 2A)，利用滅菌後之針頭沾取菌落中央黑綠色區域，透過光學顯微鏡可觀察到分生孢子形態如圖 2B 所示，為褐色、棍棒狀或長橢圓形、鏈生於分生孢子柄頂。分生孢子內具橫格及縱格，Alt-001 及 Alt-008 之孢子大小分別為長寬 20.15 ± 0.75 μm × 10.65 ± 0.29 μm 及 18.42 ± 0.66 μm × 9.41 ± 0.18 μm，長寬比平均為 1.97 及 1.95。為進一步進行菌株種的鑑定，將 Alt-001 及 Alt-008 分離株之 ITS (accession No. 分別為 KR336547 及 MG827243) 序列及 *endo*PG 基因部分序列 (accession No. 分別為 KR493348 及 MG859905)，依據 Maximum likelihood 進行類緣關係分析，結果顯示 Alt-001 及 Alt-008 均屬於 *A. alternata* (圖 3)。

A. alternata 在不同品種柑橘之病原性

為進一步瞭解 *A. alternata* 於不同柑橘品種上之病原性，本研究將 Alt-001 分離株之孢子懸浮液直接噴布於「茂谷柑」及「明尼橘柚」之嫩葉上，於接種 2 d 後，「茂谷柑」嫩葉開

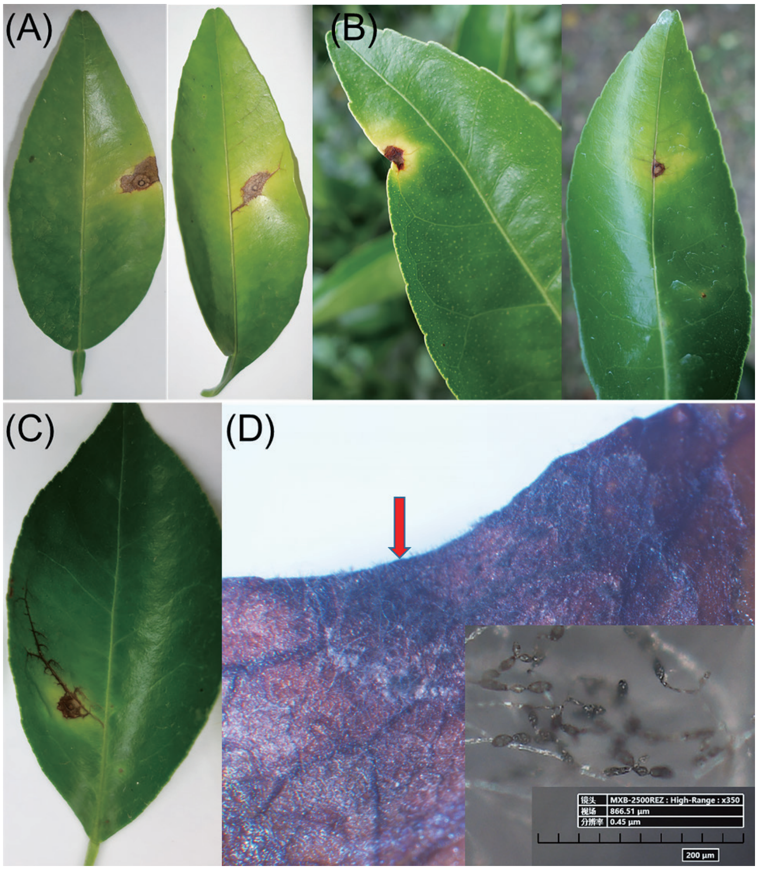

圖 1. 柑橘褐斑病在葉片上病徵。(A)「佛利蒙柑」；(B)「茂谷柑」；(C)「帝王柑」；(D) 罹病葉片上產孢情形 (紅色箭頭表示、150×)。

Fig. 1. Brown spot symptoms appeared on leaves of ‘Fremont’ (A), ‘Murcott tangor’ (B), ‘Gonggan’ (C), and the sporulation on infected leaf (D) (150×).

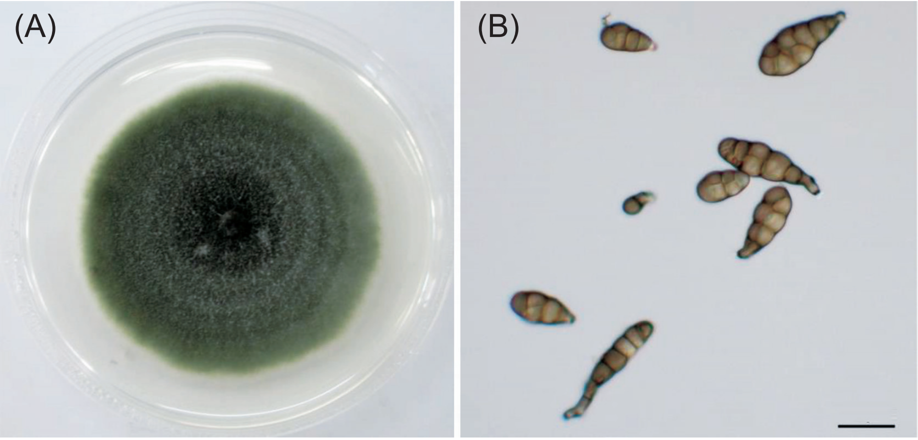

圖 2. 柑橘褐斑病菌之菌落及分生孢子形態。(A) 柑橘褐斑病菌於 PDA 培養基上於 25℃培養 8 d 後的菌落形態；(B) 分生孢子呈褐色、棍棒狀或長橢圓形，內具橫格及縱格。標尺 = 20 μm。

Fig. 2. Colony and conidia of *Alternaria alternata*. Colony of *A. alternata* on potato dextrose agar (PDA) at 25℃ for 8 d (A). Conidia were brown, clavate or oval, with transverse and longitudinal septa (B). Bar = 20 μm.

始出現黑褐色斑點病斑 (圖 4A)。接種 7 d 後，病斑逐漸擴大為似圓形褐色病斑 (圖 4B)。而當接種於「明尼橘柚」嫩葉上，則於接種 5 d 後出現黑褐色病斑 (圖 4C)，之後再進行罹病組織分離。結果顯示，所得菌株與接種病原菌菌株，其形態特徵皆相同。另將 Alt-001 分離株之孢子懸浮液，以傷口接種於「晚崙西亞」、「佛利蒙柑」、「桶柑」、「明尼橘柚」、「豔陽柑」、「茂谷柑」、「台農天王柑」及「粗皮檸檬」等 8 種不同柑橘品種離葉嫩葉葉背上，於接種 4 d 後，可在於「晚崙西亞」、「佛利蒙柑」、「桶柑」、「明尼橘柚」、「豔陽柑」、「茂谷柑」及「台農天王柑」等 7 種柑橘之葉背片上產生近圓形褐色病斑 (圖 5)，有時在病斑上可見病原菌之墨綠色菌絲 (圖 5G–5H)。但在「粗皮檸檬」嫩葉及以無菌水處理之對照葉片上，則未見病斑產生 (圖 5I、5F)。

溫度對 *A. alternata* 菌絲生長及孢子發芽之影響

Alt-001 及 Alt-008 於 10–25℃培養時，其菌絲生長隨著溫度升高而增快，於 25℃培養時，對菌絲生長最為有利，於培養後 9 d 時其平均生長直徑分別為 7.8 cm 及 6.2 cm；Alt-001 菌株於 25–35℃下生長速率遞減，至 40℃時完全不生長。Alt-008 菌株則在 30–35℃間培養時，其平均生長直徑並無顯著差異，但至 40℃培養時病原則菌絲則完全不生長 (圖 6)。而就溫度對孢子之發芽影響而言，由圖 7 顯示，兩菌株在 25–35℃之發芽率並無顯著差異。在 25℃下，Alt-001 及 Alt-008 菌株之孢子發芽率分別為 92% 及 81%，而 10℃及 40℃下孢子則完全不發芽。

化學藥劑對柑橘褐斑病菌菌絲生長之影響

將 10 種供試藥劑添加於 PDA 培養基，測試各藥劑對 *A. alternata* 菌絲生長之抑制情形，結果如表 1 所示。對 Alt-001 菌株而言，於 10 mg a.i. L^{-1} 有效成分藥劑濃度下，快得寧對其菌絲生長抑制效果最佳，可達 99.2% 抑制率，其次為易胺座及扶吉胺，其對菌絲生長抑制率分別為 68.3% 及 65.8%。當藥劑濃度提高至 100 mg a.i. L^{-1} 時，仍以此 3 個藥劑對菌絲生長有較明顯抑制作用，其他測試藥劑對 Alt-001 菌絲生長抑制率均在 50% 以下。而就 Alt-008 而言，於 10 mg a.i. L^{-1} 有效成分藥劑濃度下，則以快得寧及扶吉胺對其菌絲生長抑制效果最佳，其菌絲生長抑制率分別為 76.7% 及 73.9%，其次為易胺座 61.6 %。當藥劑濃度提高至 100 mg a.i. L^{-1} 時，仍以此 3 個藥劑對菌絲生長有較明顯抑制作用，其他測試藥劑對

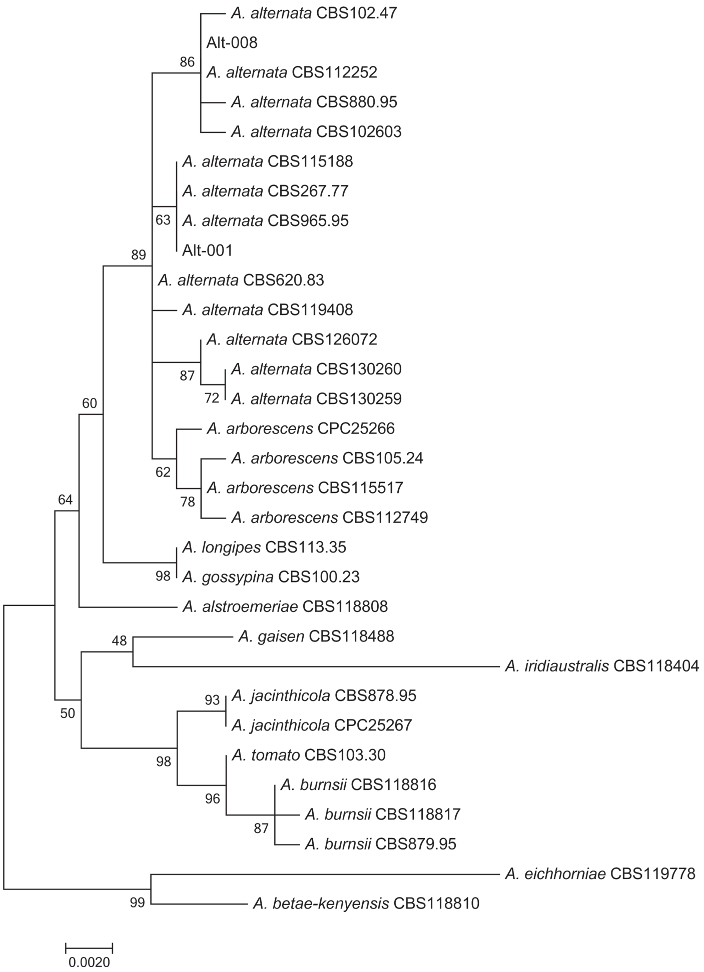

圖 3. 利用 Maximum likelihood 的方法分析 internal transcribed spacer (ITS) 及 endopolygalacturonase (*endo*PG) 序列，以進行建構 *Alternaria* spp. 類緣關係之系統樹。

Fig. 3. Phylogenetic tree based on the internal transcribed spacer (ITS) and endopolygalacturonase (*endo*PG) genes sequences of 12 species, 31 isolates of *Alternaria* spp.

圖 4. 柑橘褐斑病病原菌 (*Alternaria alternata* Alt-001) 分別接種於 (A、B)「茂谷柑」及 (C)「明尼橘柚」葉片上之病徵表現。

Fig. 4. Brown spot symptoms appeared on leaves after inoculation of *Alternaria alternata* Alt-001. Symptoms of dotted to elongated dark brown spots were observed on ‘Murcott tangor’ 2 d (A) and 7 d (B) post inoculation (dpi), respectively. Symptoms of elongated dark brown spots were observed on ‘Minneola’ 5 dpi (C).

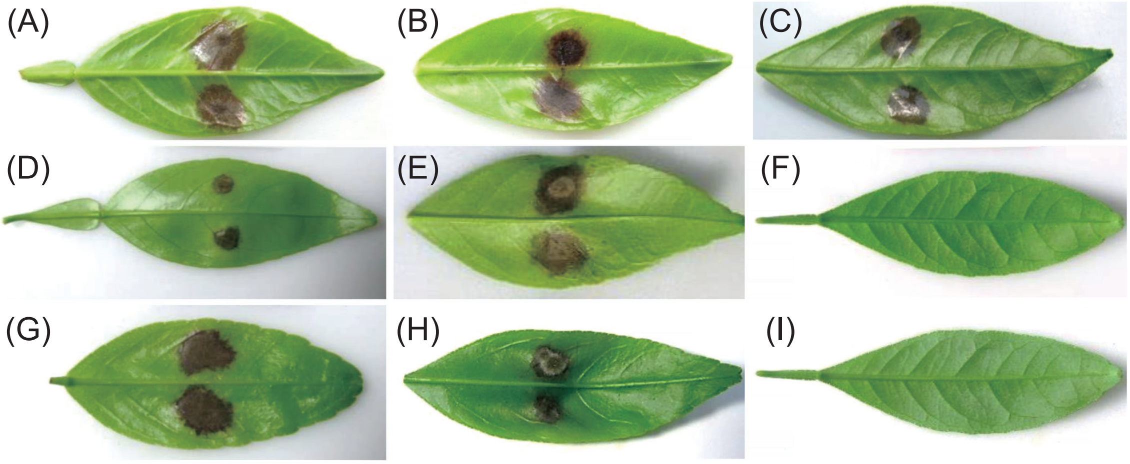

圖 5. 柑橘褐斑病病原菌 (*Alternaria alternata* Alt-001) 分別接種於 (A)「晚崙西亞」、(B)「佛利蒙柑」、(C)「桶柑」、(D)「明尼橘柚」、(E)「豔陽柑」、(F)「粗皮檸檬」、(G)「茂谷柑」及 (H)「台農天王柑」離葉嫩葉葉片上之病徵表現，以無菌水接種為 (I) 對照組「粗皮檸檬」。

Fig. 5. Brown spot symptoms caused by inoculating *Alternaria alternata* Alt-001 through wounds on detached young leaves of 'Valencia Orange' (A), *Citrus reticulata* Blanco, 'Fremont' (B), 'Tankan' (C), 'Minneola tangor' (D), 'Sunburst' (E), 'Rough lemon' (F), 'Murcott tangor' (G), or 'Tainung Giant' (H) 4 dpi, and leaf of 'Rough lemon' inoculated with water as control (I).

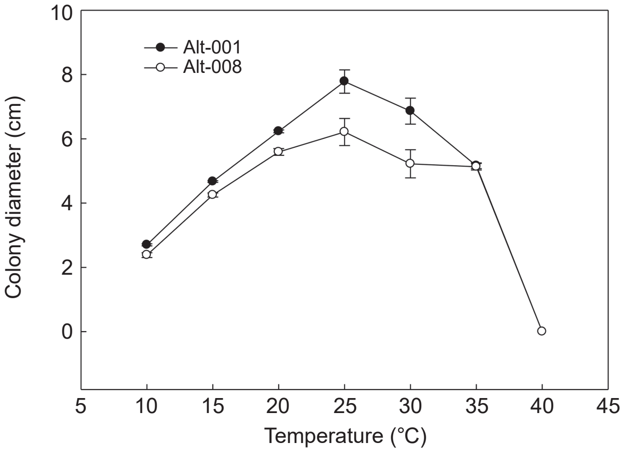

圖 6. 不同溫度對柑橘褐斑病菌 *Alternaria alternata* Alt-001 及 Alt-008 分離株病原菌菌絲生長之影響 [在 potato dextrose agar (PDA) 平板培養 9 d]。

Fig. 6. Effect of temperature on the mycelial growth of *Alternaria alternata* Alt-001 and Alt-008 isolates cultured on potato dextrose agar (PDA) plates for 9 d.

Alt-008 菌絲生長抑制率均在 50% 以下。

化學藥劑對柑橘褐斑病菌孢子發芽之影響

測試 7 種藥劑對 *A. alternata* 病原菌孢子發芽之影響， 結果如表 2 所示，快得寧及扶吉胺於 1 mg a.i. L^{-1} 有效成分濃度處理時，Alt-001 及 Alt-008 菌株之孢子發芽率均在 5.0% 或以下，對於孢子發芽具有極佳的抑制性。當濃度提高至 100 mg a.i. L^{-1} 有效成分濃

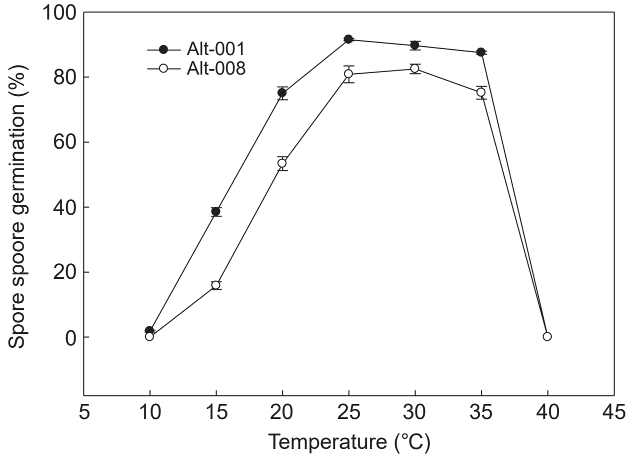

圖 7. 不同溫度對柑橘褐斑病菌 *Alternaria alternata* Alt-001 及 Alt-008 分離株孢子發芽之影響 [在 potato dextrose agar (PDA) 平板培養 7d]。

Fig. 7. Effect of temperature on spore germination of *Alternaria alternata* Alt-001 and Alt-008 isolates cultured on potato dextrose agar (PDA) plates for 7 d.

表 1. 不同藥劑對 *Alternaria alternata* Alt-001 及 Alt-008 分離株菌絲生長之影響。

Table 1. Effects of different fungicides on mycelial growth of *Alternaria alternata* Alt-001 and Alt-008 isolates.

	Inhibition (%)[z]					
	Alt-001			Alt-008		
Fungicide	1 mg a.i. L^{-1}	10 mg a.i. L^{-1}	100 mg a.i. L^{-1}	1 mg a.i. L^{-1}	10 mg a.i. L^{-1}	100 mg a.i. L^{-1}
15.0% Imibenconazole (WP[y])	24.2 b[x]	68.3 b	81.9 b	56.1 a	61.6 b	66.2 b
33.5% Oxine-copper (SC)	7.5 de	99.2 a	99.2 a	16.4 d	76.7 a	89.3 a
70.0% Thiophanate methyl (WP)	0.0 e	0.0 e	3.3 i	0.0 e	0.0 e	3.8 ef
72.0% Bordeaux mixture (WP)	6.9 e	2.2 e	0.0 i	1.7 e	2.7 e	0.0 f
33.0% Mancozeb (SC)	5.8 e	36.9 c	36.4 d	0.2 e	10.4 d	48.1 c
81.3% Kasugamycin + copper oxychloride (WP)	16.1 c	14.7 d	15.0 h	1.7 e	7.2 de	4.6 ef
25.0% Azoxystrobin (SC)	15.0 cd	6.1 e	20.8 gh	34.7 c	30.4 c	34.0 d
39.5% Fluazinam (SC)	48.1 a	65.8 b	73.1 c	50.0 b	73.9 a	85.3 a
61.4% Copper (II) Hydroxide (WG)	14.6 cd	14.6 d	25.0 fg	0.0 e	4.5 de	0.2 f
27.1% Tribasic copper sulfate (SC)	20.8 bc	32.6 c	42.8 d	0.0 e	0.0 e	6.9 e
LSD (P = 0.05)	7.6	7.7	4.2	4.1	7.5	5.6

[z] Inhibition (%) = [(Diameter of mycelial growth on PDA without fungicide – diameter of mycelial growth on PDA with fungicides)/ diameter of mycelial growth on PDA without fungicide] × 100%. PDA: potato dextrose agar.
[y] WP: wettable powders; SC: suspension concentrates.
[x] Means within a column followed by the same letter are not significantly different at 5% by least significant difference (LSD) test.

度時，則鋅錳乃浦可完全抑制兩菌株之孢子發芽，而氫氧化銅僅能抑制 Alt-001 之孢子發芽，Alt-008 則仍有 73.5% 發芽率，其他測試藥劑在 100 mg a.i. L^{-1} 有效成分藥劑濃度處理下對 Alt-001 及 Alt-008 之孢子發芽率影響均不顯著。

藥劑於柑橘葉片施用不同時間後對 *A. alternata* 分離株孢子發芽之影響

將測試藥劑噴布於溫室內「茂谷柑」葉片後，於不同施用時間後將葉片採回實驗室，再

表 2. 殺菌劑對 *Alternaria alternata* Alt-001 及 Alt-008 分離株孢子發芽之影響。

Table 2. Effects of fungicides on spore germination of *Alternaria alternata* Alt-001 and Alt-008 isolates.

	Spore germination (%)[z]					
	1 mg a.i. L^{-1}		10 mg a.i. L^{-1}		100 mg a.i. L^{-1}	
Fungicide	Alt-001	Alt-008	Alt-001	Alt-008	Alt-001	Alt-008
81.3% Kasugamycin + copper oxychloride (WP[y])	96.7 a[x]	95.5 a	96.8 a	95.3 a	97.5 a	96.5 a
80% Mancozeb (WP)	93.8 b	63.0 c	44.7 d	16.0 c	0.0 c	0.0 e
77% Copper (II) Hydroxide (WP)	96.5 a	91.3 b	76.5 c	84.3 b	0.0 c	73.5 d
72% Bordeaux mixture (WP)	96.3 a	96.0 a	83.2 b	94.8 a	45.0 b	86.5 c
70% Thiophanate methyl (WP)	96.8 a	94.8 a	96.5 a	94.0 a	97.0 a	94.0 b
40% Oxine-copper (WP)	0.0 d	0.0 e	0.0 e	0.0 d	0.0 c	0.0 e
39.5% Fluazinam (SC)	3.3 c	5.0 d	1.3 e	0.0 d	0.0 c	0.0 e
LSD	1.3	3.4	3.2	5.0	2.0	2.2

[z] Spore germination (%) = [(No. of spore germinate on water with fungicide/No. of spore germinate on water without fungicide)] × 100%.
[y] WP: wettable powders; SC: suspension concentrates.
[x] Means within a column followed by the same letter are not significantly different at 5% by least significant difference (LSD) test.

將 Alt-001 菌株之孢子懸浮液直接滴在葉片上，並進行孢子發芽率之計算，結果如表 3 所示。Alt-001 孢子懸浮液在鋅錳乃浦、氫氧化銅、快得寧及波爾多液噴布處理後 3 d 之葉片上，其孢子發芽率分別為 7.1、7.5、8.0 及 15.5%，與在對照處理水的葉片 99.3% 的發芽率均有顯著性差異，而測試之孢子若在噴布甲基多保淨之葉片上，則仍有高達 99.3% 的孢子發芽率，與對照無差異性。當藥劑處理後 21 d 再將葉片採回測試其藥效時，結果顯示，Alt-001 分離株孢子在快得寧、鋅錳乃浦、波爾多液及氫氧化銅等 4 種藥劑處理的葉片上皆僅有 16.0% 以下的發芽率，對於其孢子之發芽具有極長時間的抑制性。

討論

Alternaria spp. 可為腐生、內生或具有病原性的寄生菌，寄主範圍極廣 (Chung 2012)，

表 3. 施用藥劑後於不同時間下對 *Alternaria alternata* Alt-001 分離株孢子發芽之影響。

Table 3. Effects of fungicides on spore germination of *Alternaria alternata* Alt-001 in different period of time after treatment.

	Spore germination (%)[z]				
Fungicide	3 d	5 d	7 d	14 d	21 d
33.5% Oxine-copper (SC[y]) 500×	8.0 c[x]	4.0 c	6.0 c	4.4 d	4.9 d
72.0% Bordeaux mixture (WP) 500×	17.4 b	4.8 c	34.1 b	12.6 c	15.3 c
70.0% Thiophanate methyl (WP) 1,000×	99.3 a	99.6 a	99.5 a	99.7 a	99.0 a
33.0% Mancozeb (SC) 500×	7.1 c	5.1 c	9.4 c	10.4 cd	6.9 d
61.4% Copper(II) hydroxide (WP) 800×	7.5 c	6.4 c	6.4 c	5.1 d	5.6 d
39.5% Fluazinam (SC) 2,000×	15.5 b	32.3 b	35.3 b	67.1 b	48.9 b
Control	99.3 a	99.5 a	99.4 a	99.8 a	99.8 a
LSD (P = 0.05)	3.5	4.1	5.9	6.2	6.8

[z] Spore germination (%) = [(No. of spore germinate on water with fungicide/No. of spore germinate on water without fungicide)] × 100%.
[y] SC: suspension concentrates; WP: wettable powders.
[x] Means within a column followed by the same letter are not significantly different at 5% by least significant difference (LSD) test.

在台灣已知寄主有馬鈴薯、番茄、甘藍、蘿蔔、辣椒、煙草等 (Hsu *et al*. 2002)。以 *A. alternata* 而言，寄主作物可達 100 種以上，其可產生寄主專一性毒質，其毒質結構從簡單的低分子量化合物至環狀胜肽均有 (Ohtani *et al*. 2009)。由 *A. alternata* 引起柑橘褐斑病主要有兩個病原型，一為 tangerine pathotype (雜柑型)，影響 tangerine cultivars 及其雜交種 (hybrids)，另一為粗皮檸檬型 (rough lemon pathotype)，影響「粗皮檸檬」及「廣東檸檬」。Tangerine pathotype 及 rough lemon pathotype 在寄主上產生之毒質不同，分別為 ACT 毒質及 ACRL 毒質 (Kohmoto *et al*. 1979; Nishimura & Kohmoto 1983; Kohmoto *et al*. 1991)。由前人研究指出，幾乎所有的 tangerine cultivars 及其雜交種 (hybrids) 包含「明尼橘柚」(‘Minneola’)、「奧蘭多橘柚」(‘Orlando’, *Citrus paradisi* × *C. reticulata*)、「茂谷柑」(‘Murcott tangor’, *C. reticulata* × *Citrus sinensis*)、雜交柑「新星」(‘Nova’) 、‘Page’、‘Farchild’、‘Fortune’ 與「豔陽柑」(‘Sunburst’) 等對 tangerine pathotype (雜柑型) 均為感病品種 (susceptible cultivars) (Solel & Kimchi 1997; Peever *et al*. 2000; Timmer *et al*. 2000b; Reis *et al*. 2007)；而「溫州蜜柑」(‘Satsuma mandarin’, *Citrus unshiu*)、「克里曼丁桔」(‘Clementine’, *Citrus clementina*)、「橙」(*C. sinensis*)、「檸檬」(*Citrus limon*) 及「金棗」(*Citrus margarita*) 等品種對 *A. alternata* Tangerine pathotype 較具有抗性 (resistance) (Kohmoto *et al*. 1991; Reis *et al*. 2007; Khanchouch *et al*. 2017)。本研究所分離的 Alt-001 菌株，在進行病原性接種時發現對「茂谷柑」、「明尼橘柚」、「豔陽柑」、「晚崙西亞」、「桶柑」、「佛利蒙柑」及「台農天王柑」均有病原性，但在粗皮檸檬上則不具有病原性。且以病勢進展看來，又以對「豔陽柑」、「茂谷柑」及「台農天王柑」之病原性較強。顯示本研究所分離的 *A. alternata* 菌株應為產生 ACT 毒質的 tangerine type，對台灣目前主要高單價柑橘栽培品種如「茂谷柑」及新興品種「天王柑」等具有威脅性，應注意防治。

有關柑橘褐斑病感染之條件，根據 Timmer *et al*. (1998, 2000a) 研究指出，*A. alternata* 褐斑病菌分生孢子為 air-borne，當相對濕度大於 85% 以上，本菌之分生孢子即有可能在樹上罹病組織或是地上罹病落葉產生，再藉由風或雨水傳播。由田間試驗研究報告顯示，有以下幾個指標可做為 *A. alternata* 褐斑病菌可能發生之判斷因子：(1) 日降雨量大於 2.5 mm；(2) 葉面游離水持續維持 10 h 以上；(3) 日平均溫度介於 20–28℃ (Timmer *et al*. 2000a)。本研究所分離的 *A. alternata* Alt-001 及 Alt-008 分離株，20–35℃均可生長，25℃為其菌絲最適生長溫度，25–30℃為孢子發芽最適溫度；以 2015 年 3 月及 4 月台中的氣象站之資料 (http://www.cwb.gov.tw/V7/climate/agri/agri_month.htm) 顯示，其溫度範圍分別為 13.0–34.7℃ (月平均 20.7℃) 及 12.4–34.1℃ (月平均 24.4℃)；每日降雨量範圍分別為 0–7.4 mm 及 0–25.3 mm；2016 年 3 月及 4 月其溫度範圍分別為 9.4–28.9℃ (月平均 18.3℃) 及 17.3–33.2℃ (月平均 24.9℃)、每日降雨量範圍分別為 0–50 mm 及 0–38 mm；2017 年 3 月及 4 月其溫度範圍分別為 12.9–30.2℃ (月平均 20.2℃) 及 11.9–33.7℃ (月平均 23.2℃)、每日降雨量範圍分別為 0–11 mm 及 0–27.5 mm，以上 3 年資料顯示，由於 3–4 月平均溫度 20℃以上，而單日最高降雨量有時高達 50 mm，導致游離水在葉片上的存留時間拉長，適合病原菌之侵染，導致本病害之發生。因此，台灣栽培柑橘之農友仍應特別注意春天萌新梢時期褐斑病之防治。

有關柑橘褐斑病的防治，目前在國內並無推薦藥劑，本研究以推薦在柑橘其他真菌性病害之藥劑，進行柑橘褐斑病菌絲生長及孢子發芽之影響測試。試驗結果顯示，快得寧或扶吉胺於 10 mg a.i. L^{-1} 有效成分濃度可有效抑制 *A. alternata* 菌絲生長及孢子發芽，但在溫室試驗中可發現快得寧的保護效果及保護時間更優於扶吉胺；在室內孢子藥劑試驗中，鋅錳乃浦在 100 mg a.i. L^{-1} 有效成分濃度下對 *A. alternata* 之孢子發芽具有良好抑制效果，在溫室試驗中亦與快得寧相同呈現極佳的保護力。氫氧化銅對 *A. alternata* Alt-001 分離株之孢子發芽具有極佳抑制效果，但對 *A. alternata* Alt-008 分離株之孢子發芽抑制效果不佳，或與菌株間對藥

劑忍受性不同有關，此仍有待收集更多的菌株後再做證明，但此銅劑在溫室試驗中與快得寧及鋅錳乃浦均呈現極佳保護力，三者之間無顯著性差異。另外，市售波爾多液於 100 mg a.i. L^{-1} 有效成分濃度下對 *A. alternata* Alt-001 及 Alt-008 分離株之孢子發芽抑制效果不佳，但在溫室試驗中，葉片噴布波爾多液 21 d 後，*A. alternata* 孢子在葉片上之發芽率仍相當低，具有很好的保護效果，兩試驗結果迴然不同之原因在於有效濃度的差異性。由於進行室內藥劑試驗時，超過 100 mg a.i. L^{-1} 有效成分濃度之波爾多液因藥劑內顆粒太多，導致無法觀察孢子發芽，因此在進行室內藥劑試驗時僅能做到 100 mg a.i. L^{-1}，然在進行溫室試驗時，依推薦倍數進行稀釋，其噴布有效成分濃度為 1,440 mg a.i. L^{-1}，與室內藥劑試驗所使用之濃度高出 10 倍以上，故具有良好抑制孢子發芽效果。上述快得寧、氫氧化銅及波爾多液皆為銅劑，其作用機制為銅鹽可使細胞原生質凝固或銅離子 (Cu^{2+}) 被吸附於細胞表面後，與細胞壁或細胞膜之幾丁質 (或蛋白質) 的 H^+、Mg^{2+}、K^+ 等離子置換，導致細胞過度氧化及破壞酵素系統，阻礙細胞之正常生理作用，而使孢子死亡或抑制發芽 (Tzeng 2015)。而鋅錳乃浦屬於有機硫磺劑，其作用機制為可與多種酵素之 SH 基作用，破壞脂質代謝、呼吸作用與 adenosine triphosphate (ATP) 產生 (Tzeng 2015)，均為保護性藥劑，惟因本病害只感染嫩葉，故建議於新梢抽出 1/2 前即應噴藥保護，並於完全抽出後應再噴藥保護一次，避免因新梢長大，葉面上藥劑覆蓋率不佳，導致防治效率減低。扶吉胺雖同時可抑制菌絲生長及孢子發芽，但在本研究中顯示其噴布在葉片後，對 *A. alternata* 孢子發芽抑制之藥效期較短，在使用上可著重於抑制病原菌菌絲生長期使用。此外，國外研究 (Azevedo *et al*. 2015) 顯示潛葉蛾造成之傷口亦會助長褐斑病菌之侵染，因此在新梢期應一併進行潛葉蛾之防治。

由本研究結果顯示，台灣氣候條件適合 *A. alternata* 柑橘褐斑病菌之侵染，而所種植的栽培品種有許多亦屬於感病品種，如「茂谷柑」、「帝王柑」、「佛利蒙柑」及「天王柑」等。爰在柑橘春梢期尤應注意防治，避免產量損失。同時避免施用過量氮肥產生過多新梢、避免過度灌溉以及加強修剪促進通風，以免游離水存在之時間過久，有助於孢子發芽侵染。另外，應於下雨前選擇使用抑制病原孢子發芽較佳之藥劑如快得寧、氫氧化銅及波爾多液等銅劑進行春梢期保護措施，雨季後宜選擇抑制菌絲生長較為有效之藥劑如扶吉胺或快得寧，俾降低田間病原菌密度，以達到本病害防治目的。

誌謝

本研究之進行承蒙嘉義農業試驗分所園藝系陳祈男助理研究員提供不同品種柑橘葉片進行病原性接種，使得試驗順利完成，在此一併致謝。

引用文獻

Azevedo, F. A., I. B. Martelli, D. A. Polydoro, C. A. Pacheco, E. H. Schinor, and M. Batianel. 2015. Positive relationship between citrus leaf miner and alternaria brown spot. Cienc. Rural 45:1160–1163.

Chung, K. R. 2012. Stress response and pathogenicity of the necrotrophic fungal pathogen *Alternaria alternata*. Scientifica 2012:635431. doi:10.6064/2012/635431

Cobb, N. A. 1903. Letters on the diseases of plants-*Alternaria* of the citrus tribe. Agric. gaz. N.S.W. 14:955–986.

Elena, K. 2006. Alternaria brown spot of Minneola in Greece; evaluation of citrus species susceptibility. Eur. J. Plant Pathol. 115:259–262.

Garganese, F., L. Schena, I. Siciliano, M. I. Prigigallo, D. Spadaro, A. De Grassi, A. Ippolito, and S. M. Sanzani. 2016. Characterization of citrus-associated *Alternaria* species in Mediterranean areas. PLOS ONE 11(9):e0163255. doi:10.1371/journal.pone.0163255

Hsu, S. T., T. T. Chang, C. A. Chang, J. L. Tsai, and T. T. Tsay. 2002. List of Plant Diseases in Taiwan. 4th ed. Taiwan Phytopathology Society. Taichung, Taiwan. 386 pp. (in Chinese)

Khanchouch, K., A. Pane, A. Chriki, and S. O. Cacciola. 2017. Major and emerging fungal diseases of *citrus* in the mediterranean region. p.3–29. *in*: Citrus Pathology. (Harsimran, G. and G. Harsh, eds.) IntechOpen. Rikeja, Croatia. 220 pp.

Kohmoto, K., K. Akimitsu, and H. Otani. 1991. Correlation of resistance and susceptibility of citrus to *Alternaria alternata* with sensitivity to host-specific toxins. Phytopathology 81:719–722.

Kohmoto, K., R. P. Scheffer, and J. O. Whiteside. 1979. Host-selective toxins from *Alternaria citri*. Phytopathology 69:667–671.

Marín, J. E., H. S. Fernández, N. A. Peres, M. Andrew, T. L. Peever, and L. W. Timmer. 2006. First report of alternaria brown spot of citrus caused by *Alternaria alternata* in Peru. Plant Dis. 90:686.

Ni, H. F., C. W. Huang, and H. R. Yang. 2015. First report of citrus alternaria brown spot caused by *Alternaria alternata* in Taiwan. Plant Dis. 99:1864.

Nishimura, S. and K. Kohmoto. 1983. Host-specific toxins and chemical structures from *Alternaria* species. Annu. Rev. Phytopathol. 21:87–116.

Ohtani, K., T. Fukumoto, S. Nishimura, Y. Miyamoto, K. Gomi, and K. Akimitsu. 2009. Alternaria pathosystems for study of citrus diseases. Tree For. Sci. Biotechnol. 3:108–115.

Peever, T. L., L. Olsen, A. Ibañez, and L. W. Timmer. 2000. Genetic differentiation and host specificity among populations of *Alternaria* spp. causing brown spot of grapefruit and tangerine × grapefruit hybrids in Florida. Phytopathology 90:407–414.

Peever, T. L., L. Carpenter-Boggs, L. W. Timmer, L. M. Carris, and A. Bhatia. 2005. Citrus black rot is caused by phylogenetically distinct lineages of *Alternaria alternata*. Phytopathology 95:512–518.

Peres, N. A. R., J. P. Agostini, and L. W. Timmer. 2003. Outbreaks of alternaria brown spot of citrus in Brazil and Argentina. Plant Dis. 87:750.

Reis, R. F., T. F. de Almeida, E. S. Stuchi, and A. de Goes. 2007. Susceptibility of citrus species to *Alternaria alternata*, the causal agent of the alternaria brown spot. Sci. Hortic. 113:336–342.

Reis, R. F., A. de Goes, S. N. Mondal, T. Shilts, F. C. Brentu, and L. W. Timmer. 2006. Effect of lesion age, humidity, and fungicide application on sporulation of *Alternaria alternata*, the cause of brown spot of tangerine. Plant Dis. 90:1051–1054.

Solel, Z. 1991. Alternaria brown spot on Minneola tangelos in Israel. Plant Pathol. 40:145–147.

Solel, Z. and M. Kimchi. 1997. Susceptibility and resistance of citrus genotypes to *Alternaria alternata* pv. *citri*. J. Phytopathol. 145:389–391.

Solel, Z., Y. Oren, and M. Kimchi. 1997. Control of alternaria brown spot of Minneola tangelo with fungicides. Crop Prot. 16:659–664.

Swart, S. H., M. J. Wingfield, W. J. Swart, and G. C. Schutte. 1998. Chemical control of alternaria brown spot on Minneola tangelo in South Africa. Ann. Appl. Biol. 133:17–30.

Timmer, L. W., Z. Solel, T. R. Gottwald, A. M. Ibañez, and S. E. Zitko. 1998. Environmental factors affecting production, release, and field populations of conidia of *Alternaria alternata*, the cause of brown spot of citrus. Phytopathology 88:1218–1223.

Timmer, L. W., H. M. Darhower, S. E. Zitko, T. L. Peever, A. M. Ibáñez, and P. M. Bushong. 2000a. Environmental factors affecting the severity of Alternaria brown spot of citrus and their potential use in timing fungicide applications. Plant Dis. 84:638–643.

Timmer, L. W., Z. Solel, and M. Orozco-Santos. 2000b. Alternaria brown spot of mandarins. p.19–20. *in*: Compendium of Citrus Diseases. 2nd ed. (Timmer, L. W., S, M. Garnsey, and J. H. Graham, eds.) American Phytopathological Society Press, St. Paul, MN. 92 pp.

Tsay, Y. P., B. K. Tong, B. J. An, A. S. Jheng, H. J. Su, T. C. Deng, and T. T. Tsay. 2002. Citrus disease. p. 176–280. *in*: Plant Protection 9-Compendium of Citrus Diseases and Pests. (Zou, H. J., C. F. Yan, and M. J. Jheng, eds.) Bureau of Animal and Plant Health Inspection and Quarantine. Taipei, Taiwan. 378 pp. (in Chinese)

Tzeng, D. S. 2015. Pharmacology and application of pesticides-fungicide. National Chung Hsing University, Taichung, Taiwan. 166 pp. (in Chinese)

Vicent, A., J. Armengol, R. Sales, J. Garcia-Jiménez, and F. Alfaro-Lassala. 2000. First report of alternaria brown spot of citrus in Spain. Plant Dis. 84:1044.

White, T. J., T. D. Bruns, S. B. Lee, and J. W. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. p.315–322. *in*: PCR Protocols: A Guide to Methods and Applications. (Innis, M. A., D. H. Gelfand, J. J. Sninsky, and T. J. White, eds.) Academics Press, San Diego, CA. 482 pp.

Whiteside, J. 1976. A newly recorded Alternaria-induced brown spot disease on Dancy tangerines in Florida. Plant Dis. Rep. 60:326–329.

Physiological Characteristics, Pathogenicity and Fungicide Screening of Citrus Alternaria Brown Spot Disease Caused by *Alternaria alternata*

Chiao-Wen Huang[1], Chao-Jung Wu[2], Hong-Ren Yang[3], Su-Yu Lai[4], and Hui-Fang Ni[5,*]

Abstract

Huang, C. W., C. J. Wu, H. R. Yang, S. Y. Lai, and H. F. Ni. 2018. Physiological characteristics, pathogenicity and fungicide screening of citrus alternaria brown spot disease caused by *Alternaria alternata*. J. Taiwan Agric. Res. 67(4):387–400.

Alternaria brown spot of citrus (*Citrus reticulata*) was found in orchards of 'Murcott', 'Fremont', and 'Gonggan' citrus in Taiwan during 2012–2013. This disease seriously affected the growth of citrus in spring shoot period. The objective of this study is to investigate the physiological characteristics and pathogenicity of *Alternaria alternata*, the causal agent of the brown spot disease, and further screen the effectiveness of fungicides. Seven cultivars included 'Murcott', 'Minneola', 'Tankan', 'Fremont', 'Valencia', 'Sunburst', and 'Tainung Giant'. Results showed that brown-to-black lesions appeared on the detached young leaves inoculated through wounds, but no symptom appeared on rough lemon after wound inoculation. The optimal temperature for mycelial growth and spore germination of *A*. *alternata* strain Alt-001 and Alt-008 was 25℃ and 25–30℃, respectively. Mycelial growth was effectively inhibited by 10 mg a.i. L^{-1} oxine-copper, the inhibition rate of 99.2% was measured by screening. Spore germination of *A*. *alternata* was inhibited by oxine-copper, copper(II) hydroxide, mancozeb, and fluazinam *in vitro*. Futhermore, spore germination of *A*. *alternata* was continuously inhibited by oxine-copper, copper(II) hydroxide, and mancozeb in greenhouse 21 d after fungicide treatment. These fungicides have been recommended to control other fungal diseases of citrus. Therefore, it is suggested that they could also be used to control Alternaria brown spot of citrus in term of efficacy.

Key words: Citrus (*Citrus reticulata*), Alternaria brown spot, *Alternaria alternata*, Fungicides screening.

Received: April 20, 2018; Accepted: June 6, 2018.
[*] Corresponding author, e-mail: hfni@dns.caes.gov.tw
[1] Assistant Research Fellow, Plant Pathology Division, Taiwan Agricultural Research Institute, Taichung, Taiwan, ROC.
[2] Assistant Research Fellow, Department of Plant Protection, Chiayi Agricultural Experiment Branch, Taiwan Agricultural Research Institute, Chiayi, Taiwan, ROC.
[3] Research Fellow and Director, Chiayi Agricultural Experiment Branch, Taiwan Agricultural Research Institute, Chiayi, Taiwan, ROC.
[4] Research Assistant, Department of Plant Protection, Chiayi Agricultural Experiment Branch, Taiwan Agricultural Research Institute, Chiayi, Taiwan, ROC.
[5] Associate Research Fellow and Head, Department of Plant Protection, Chiayi Agricultural Experiment Branch, Taiwan Agricultural Research Institute, Chiayi, Taiwan, ROC.

台灣農業研究 (J. Taiwan Agric. Res.) 67(4):401–413 (2018)
DOI:10.6156/JTAR.201812_67(4).0008

研究報告

植物生長調節劑與兩階段培養對薑組織培養苗增殖與生長之影響

陳威臣 [1]　曹進義 [1]　吳姿穎 [2]　夏奇鋹 [3,*]

摘要

陳威臣、曹進義、吳姿穎、夏奇鋹。2018。植物生長調節劑與兩階段培養對薑組織培養苗增殖與生長之影響。台灣農業研究 67(4):401–413。

本研究利用「廣東薑」(*Zingiber officinale* 'Guang Dong') 與「竹薑」(*Z. officinale* 'Chu') 根莖之新生芽體為培植體，建立其組織培養苗大量繁殖方式。切取長約 1 cm 之新生根莖芽體 (rhizome bud) 為培植體，先利用 75% 酒精處理 30 s 後，再以 0.6% NaOCl 消毒 15 min，培養於含有 0.4 mg L^{-1} 苯甲基腺嘌呤 (6-benzylaminopurine; BA) 之 B5 固態培養基中 12 wk 後，建立無菌培養瓶苗。將直徑約 5 mm 之「廣東薑」與「竹薑」組培根莖培養於含有 0.5–2.0 mg L^{-1} BA 與 0.1 mg L^{-1} 奈乙酸 (α-naphthalene acetic acid; NAA) 之 B5 固態培養基 8 wk 後，兩品種每個培植體平均可誘得高約 1.8–2.6 cm 之組培苗 4.8–6.0 株，且瓶苗具有良好之根系；提高 BA 濃度至 4 mg L^{-1} 雖可增加苗數，但苗高顯著降低；反之，對照組之組培苗雖然較高，但其苗數顯著較少。利用直徑約 5 mm 之組培根莖以液態培養 8 wk 後比較 BA 濃度之影響，結果顯示在 0.5–2.0 mg L^{-1} BA 處理條件下，「廣東薑」與「竹薑」之每個培植體分別誘得 7.6–7.8 芽與 7.1–7.3 芽，但苗高僅約 1.2–1.3 cm。將液態培養所得之叢生芽苗團塊，不經分割而直接繼代培養於含有 0.5 mg L^{-1} BA 與 0.1 mg L^{-1} NAA 之 B5 固態培養基 8 wk 後，若培植體源自於 0.5–2.0 mg L^{-1} BA 配合 0.1 mg L^{-1} NAA 之液態處理，「廣東薑」與「竹薑」每個培植體分別可獲得高約 2.8–3.1 cm 之組培苗 11.2–13.0 株，以及高約 4.6–5.2 cm 之組培苗 13.3–14.9 株，顯示液-固態兩階段培養可應用於薑組培苗之大量繁殖。

關鍵詞：薑、液-固態培養、苯甲基腺嘌呤、大量繁殖。

前言

薑 (*Zingiber officinale*) 源自東南亞與亞洲熱帶地區，為薑科 (Zingiberaceae) 多年生宿根草本植物，應用部位為根莖 (rhizome)，俗稱生薑或薑母。因其具有特殊辣味與香氣，自古即廣泛應用於世界各地，食用與藥用記錄可追溯 2,500 年的悠久歷史 (Mishra *et al*. 2012; Tsai *et al*. 2013; Singh *et al*. 2014; Agrahari *et al*. 2015)；美國藥物食品檢驗局 (Food and Drug Administration; FDA) 亦將薑列為一般性安全的食品添加劑 (generally recognized as safe; GRAS) (Zadeh & Kor 2014)。在藥用方面，傳統上使用薑來治療關節炎、風濕、扭傷酸痛、喉嚨痛、噁心嘔吐、高血壓、腸胃不適及發燒頭痛等症狀 (Ali *et al*. 2008; Deng & Pan 2009; Rehman *et al*. 2011; Mishra *et al*. 2012; Agrahari *et al*. 2015)。現代藥理研究證實，生薑具有清熱活血、消腫止痛、利尿解毒、緩解痠痛、健胃止吐、降血脂與膽固醇等功能；同時具有改善血液循環、降血醣、延緩衰老、護肝保胃的效果，以及抗菌、抗發炎、抗

投稿日期：2017 年 9 月 29 日；接受日期：2018 年 6 月 21 日。
* 通訊作者：hsia@tari.gov.tw
[1] 農委會農業試驗所生物技術組助理研究員。台灣 台中市。
[2] 農委會農業試驗所生物技術組研究助理。台灣 台中市。
[3] 農委會農業試驗所生物技術組研究員。台灣 台中市。

氧化及抗癌等作用 (Mishra *et al*. 2012; Singh *et al*. 2014; Zadeh & Kor 2014; Agrahari *et al*. 2015)。

近年來，產薑量最大的國家為印度，其次為中國 (Food and Agriculture Organization of the United Nations 2017)。薑在台灣約有 1,000 ha 的栽培面積，年產量約 3 萬公噸，主要產區在南投、台東、宜蘭及苗栗；栽培品種多為俗稱大指薑之「廣東薑」(又名南洋薑，*Zingiber officinale* 'Guang Dong')，其次為俗稱小指薑的「竹薑」(又名本島薑，*Z. officinale* 'Chu')；依據薑不同生育階段採收之產品可分為嫩薑、粉薑、老薑 (或稱薑母) 及種薑 (Tsai *et al*. 2013; Chen *et al*. 2014; Council of Agriculture, Executive Yuan 2017a, 2017b; Hsieh *et al*. 2017)。薑栽培以無性繁殖根莖種苗為主，除了可供選擇之品種極少外，且種薑品質難以維持，主因是受到軟腐病 (soft rot, 病原為 *Pythium myriotylum*) 與青枯病 (bacterial wilt, 病原為 *Ralstonia solanacearum*) 造成之根莖腐爛，此兩病原均可藉由種薑或田間留存傳播，因此連作薑田病害發生機率最高，嚴重影響產量與品質；次為根瘤線蟲 (root-knot nematode, *Meloidogyne incogntia*) 的危害，傳播途徑也是藉由種薑或土壤中蟲卵擴散，蟲體入侵根莖後所造成的傷口更有利其他病菌侵入 (Han 2000; Kambaska & Santilata 2009; Kavyashree 2009; Abbas *et al*. 2011; Sathyagowri & Seran 2011; Tsai *et al*. 2013)。此外，由於種薑儲存不易，因此栽種適期常面臨薑苗缺乏之困境。為解決上述問題，優質種薑量產技術之建立實屬必要。

組織培養技術常用於作物種苗繁殖，具有繁殖倍率大、繁殖週期短、可週年生產等優點，是快速量產品質均一種苗的有效方法。目前薑產業均利用塊狀根莖種植栽培，此種無性種苗之繁殖效率偏低，且易成為病害擴散之感染源，採用組織培養技術建立薑種苗繁殖體系不僅可排除田間病原汙染，更具有繁殖率高之優點，是目前無性繁殖根莖種薑所不能及 (Huang *et al*. 2004; Luo *et al*. 2006; Lincy & Sasikumar 2010; Seran 2013; David *et al*. 2016; Li 2016)。薑之微體繁殖多利用生長點 (Han 2000; Huang *et al*. 2004) 或根莖芽體 (rhizome bud) (Kavyashree 2009; Abbas *et al*. 2011; Ayenew *et al*. 2012)，培養於含有苯甲基腺嘌呤 (6-benzylaminopurine; BA) 與奈乙酸 (α-naphthalene acetic acid; NAA) 組合之培養基中以建立其組織培養繁殖體系，惟芽體繁殖倍率僅在 2−5 之間。針對薑之組織培養程序而言，則各研究報告存有差異，部分學者指出其繁殖體系須經芽體伸長與發根階段 (Han 2000; Huang *et al*. 2004; Kambaska & Santilata 2009; Abbas *et al*. 2011; Sathyagowri & Seran 2011; Ayenew *et al*. 2012)，而有些報告則指出可同時進行芽體誘導、伸長及發根而成苗 (Kavyashree 2009)。此外，液態培養可促進組培芽體增殖率，而後將叢生芽體培養於固態培養基中，即可形成發根良好的組培苗 (Lincy & Sasikumar 2010)。

本研究擬以薑根莖芽體建立微體繁殖，利用液態培養促進芽體增殖率，再將叢生芽體轉移至固態培養基之液-固態兩階段培養方式，生產不帶真菌或細菌病原之優質種苗作為生產種薑之用，提供農民更多元之選擇。

材料與方法

植物材料來源與消毒條件

本研究利用「廣東薑」與「竹薑」根莖芽體作為建立組織培養之材料。取自田間之根莖以清潔劑清洗去除表面殘土後，置於室溫約 26℃環境下誘導芽體萌發。切取長約 1–2 cm 之「廣東薑」與「竹薑」根莖新生芽體，剝去 2–3 層鞘葉後作為培植體。先以 75% 酒精溶液 (ethanol) 浸泡手搖 30 s，而後浸入 0.6% 次氯酸鈉 (NaOCl, The Clorox Co., CA. USA) 溶液，置於水平迴轉振盪器 (orbital shaker, SK-302AB, SanKuan Co., Taichung, Taiwan)，利用 80 rpm 速度振搖 15 min 進行消毒。取出後以無菌水沖洗 3 次，而後接種於含有 0.4 mg L^{-1} BA (Sigma-Aldrich Inc., St. Louis, MO, USA) 之 B5 (Gamborg *et al*. 1968) 固態培養基，用以建立薑之組織培養瓶苗。

培養基配製與試驗培養條件

培植體係培養於含有 3% 蔗糖、0–4 mg L^{-1} BA 配合 0.1 mg L^{-1} NAA (Sigma-Aldrich Inc., St. Louis, MO, USA) 之 B5 固態或液態培養基；固態培養基於加入 9 g L^{-1} 洋菜 (Difco Bacto-agar, Carolina Co., Burlington, VT, USA) 前，先以 0.1–1 N NaOH 或 HCl 將 pH 值調為 5.7 ± 0.1，液態培養基則未添加洋菜，且 pH 值調為 5.2 ± 0.1。培養基以 121℃、1.05 kg cm^{-2} 滅菌 20 min 後，冷卻備用。

固態與液態培養均是將培植體接種於內含 20 mL 培養基之 125-mL 三角瓶 (Erlenmeyer flask)；固態培養置於 26℃ ± 2℃、光照 14 h、光強度 38 μmol m^{-2} s^{-1} 的環境；液態培養除使用 10 μmol m^{-2} s^{-1} 低光照外，其餘環境條件與固態培養相同，並置於水平迴轉式振盪器，以 80 rpm 速度進行振盪培養。試驗採 3 重複，每瓶接種 3 個培植體，共接種 9 個培植體。

BA 濃度於固態或液態培養對薑組培苗增殖與生長之影響

將「廣東薑」與「竹薑」組培苗之假莖與根部切除後，取直徑約 5 mm 根莖作為培植體，培養於含不同 BA 濃度 (0、0.5、1、2、4 mg L^{-1}) 與 0.1 mg L^{-1} NAA 之 B5 固態或液態培養基。培養 8 wk 後，調查每個培植體誘導形成瓶苗數與芽數；調查時依瓶苗或芽體之生長形態分別記錄葉片展開苗 (plantlet with unfolded leaves; PL Fig. 3)、葉片未開之綠色芽 (green shoot-bud; GS) 於結果及淡黃色芽 (yellowish shoot-bud; YS) 標註的數量，三者總和為總芽數；瓶苗數或芽數除以總芽數，則表示不同形態芽苗的占比。苗高僅調查葉片展開苗，自假莖基部至頂葉之葉身基部的高度。

液-固態兩階段培養對薑組織培養苗增殖與生長之影響

利用源自上述不同 BA 濃度液態培養所得之叢生芽苗團塊作為培植體，不經分割而直接繼代培養於內含 100 mL 固態培養基之 500-mL 蘭花瓶 (Orchid Flask, Taiwan Glass Ind. Corp., Taipei, Taiwan) 中。培養基為含有 0.5 mg L^{-1} BA 與 0.1 mg L^{-1} NAA 之 B5 固態培養基，培養 8 wk 後，調查每個培植體形成之瓶苗數與高度；以具有葉片展開苗之培植體數除以總培植體數代表其成苗率。

試驗設計和統計分析

本研究採用完全逢機設計 (completely randomized design; CRD) 進行試驗。試驗所得數據若為比例資料，須先經角度轉換後再進行分析。經 SAS Enterprise Guide 7.1 (SAS Institute Inc., Cary, NC, USA) 套裝統計分析軟體進行變方分析 (analysis of variance; ANOVA)，若處理間差異顯著 ($P < 0.05$)，則以最小顯著差異性測驗 (least significant difference test; LSD) 比較各處理平均值間之差異。

結果

薑組織培養無菌培植體之建立

本研究切取長約 1–2 cm「廣東薑」新生根莖芽體 (圖 1A、1B) 為材料，剝除 2–3 層鞘葉後，以 75% 酒精與 0.6% 次氯酸鈉溶液進行兩階段消毒處理。之後接種於含有 0.4 mg L^{-1} BA 之 B5 培養基，4 wk 後即可萌發成苗 (圖 1C)。而後將小苗接種於相同組成之新鮮培養基，經 8 wk 培養後可建立薑的無菌組培苗 (圖 1D)；「竹薑」之無菌組培苗，亦以上述方法建立 (資料未列)。

固態培養下 BA 濃度對薑組培苗增殖與生長之影響

將直徑約 5 mm 之「廣東薑」與「竹薑」組培根莖，培養於含有 0.5–2.0 mg L^{-1} BA 與 0.1 mg L^{-1} NAA 之 B5 固態培養基培養 8 wk 後，兩品種在此條件下表現相類似，每個培植體平均可誘得 4.8–6.0 株組培苗，苗高約為 1.3–2.6 cm (表 1)。提高 BA 濃度至 4 mg L^{-1} 雖可增加苗數，兩品種分別獲得 7.2 與 7.7 株組培苗，但其苗高卻明顯降低，分別僅約 1.3 與 1.8 cm。反之，對照組之苗高雖然較高，分別達 2.2 與 3.2 cm，其苗數與 0.5–1.0 mg L^{-1} BA 處理組之結果相近，但明顯較 2–4 mg L^{-1} BA 處理

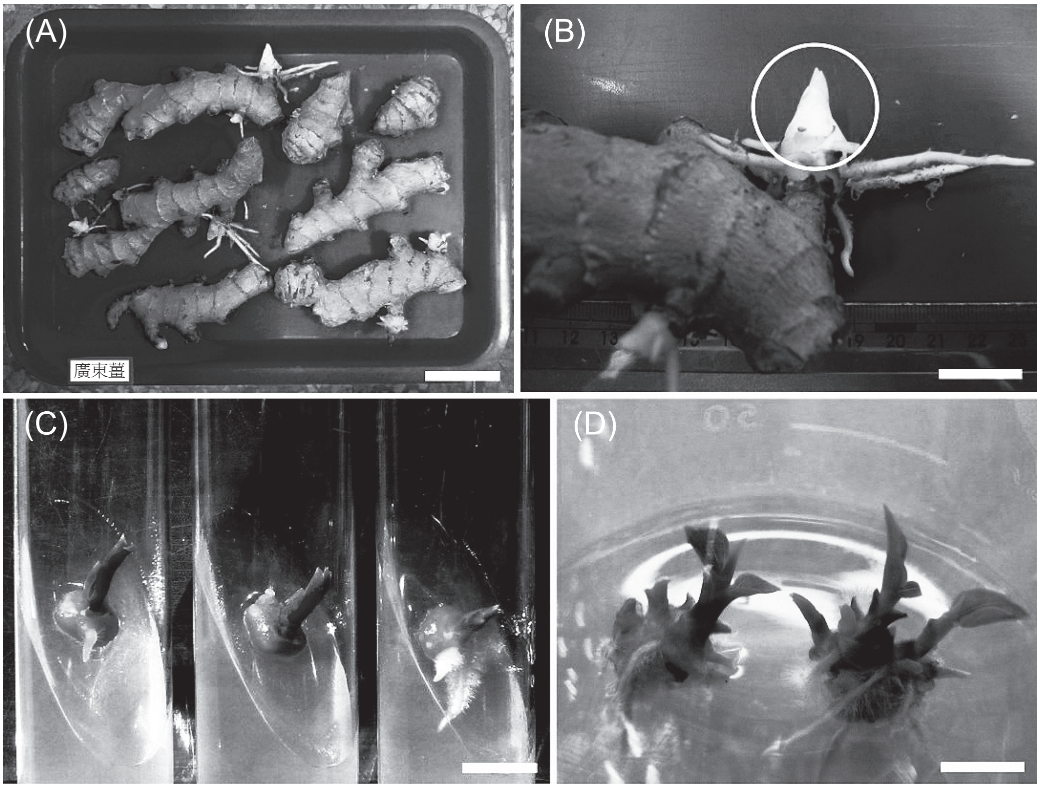

圖 1. 建立「廣東薑」組織培養初代培養。新生根莖芽體萌發於採自田間之根莖 (A, Bar = 5 cm; B, Bar = 2 cm)；根莖芽體培養於含有 0.4 mg L^{-1} BA 之 B5 固態培養基 4 wk 後，芽體生長之情形 (C, Bar = 1 cm)；將此無菌芽體繼代培養於相同組成之固態培養基達 8 wk 後，組培苗增殖與生長之情形 (D, Bar = 1 cm)。

Fig. 1. Establishment of *in vitro* culture of *Zingiber officinale* ‘Guang Dong’. New rhizome bud germinated on rhizome derived from field (A, Bar = 5 cm; B, Bar = 2 cm), *in vitro* shoot grew while rhizome bud cultured on B5 medium supplemented with 0.4 mg L^{-1} 6-benzylaminopurine (BA) for 4 wk (C, Bar = 1 cm), and multiple shoots were induced after *in vitro* shoot cultured on the same fresh medium for another 8 wk of culture (D, Bar = 1 cm).

組少，每個培植體僅獲得約 4.4 與 4.1 株組培苗 (表 1)。此外，對照組與各 BA 濃度處理之瓶苗均可發根良好 (圖 2)。

就組培苗高度而言，「廣東薑」除 8.1% 對照組組培苗高於 4 cm 外，於 0.5–2.0 mg L^{-1} BA 處理條件下之苗高占比均類似，苗高小於 2 cm 的比率約為 32.6–43.5%，而苗高介於 2–4 cm 者約為 56.5–67.4%；4 mg L^{-1} BA 處理之組培苗以低於 2 cm 的比率最高，達 71.2% (表 1)。「竹薑」結果顯示，BA 處理組之組培苗大多介於 2–4 cm，比率約為 67.9–80.1%；對照組之組培苗則以大於 4 cm 苗高比率最高，達 50.0% (表 1)。

液態培養下 BA 濃度對薑組培苗增殖與生長之影響

將直徑約 5 mm「廣東薑」與「竹薑」組培根莖培養於含 0.0–4.0 mg L^{-1} BA 與 0.1 mg L^{-1} NAA 之 B5 液態培養基中，經培養 8 wk 後所得瓶苗或芽體呈現 3 種形態，分別為葉片展開苗 (PL, 圖 3A2)、葉片未開之綠色芽 (GS, 圖 3B3) 及淡黃色芽 (YS, 圖 3A4)，其中若綠色芽與淡黃色芽高度小於 1 cm 則不予記錄。「廣東薑」與「竹薑」每個培植體分別獲得 7.6–7.8 芽與 7.1–7.3 芽，苗高僅 1.2–1.3 cm (表 2、圖 3)。將 BA 濃度提高至 4 mg L^{-1} 時，則「廣東薑」所得均為葉片未展開之綠色芽與淡黃色芽。兩

表 1. BA 濃度於固態培養下對「廣東薑」與「竹薑」組培苗增殖與生長之影響。

Table 1. Effect of BA in the solid medium on plantlet proliferation and growth of *in vitro Zingiber officinale* 'Guang Dong' and 'Chu'[z].

Plant growth regulator	No. of plantlets	Plantlet height (cm)	Percent of plantlet height (%)		
BA (mg L^{-1})			< 2 cm	2–4 cm	> 4 cm
'Guang Dong'					
0.0	4.4 ± 0.4 c[y]	2.2 ± 0.1 a	34.2 ± 11.2 b	57.6 ± 17.5 a	8.1 ± 2.1 a
0.5	5.6 ± 0.7 bc	1.8 ± 0.2 b	32.6 ± 12.6 b	67.4 ± 12.6 a	0.0 b
1.0	5.4 ± 1.1 bc	1.8 ± 0.2 b	43.5 ± 18.8 b	56.5 ± 18.8 a	0.0 b
2.0	6.0 ± 0.5 ab	1.8 ± 0.1 b	33.8 ± 11.7 b	66.2 ± 11.7 a	0.0 b
4.0	7.2 ± 0.5 a	1.3 ± 0.2 c	71.2 ± 10.6 a	28.8 ± 10.6 b	0.0 b
'Chu'					
0.0	4.1 ± 0.4 c	3.2 ± 0.3 a	19.6 ± 8.2 ab	30.4 ± 9.3 b	50.0 ± 1.1 a
0.5	4.8 ± 0.4 bc	2.6 ± 0.3 b	13.3 ± 3.1 b	80.1 ± 6.8 a	6.7 ± 1.7 b
1.0	4.9 ± 0.2 bc	2.2 ± 0.2 bc	22.8 ± 5.0 ab	77.2 ± 6.4 a	0.0 c
2.0	6.0 ± 0.6 b	2.1 ± 0.1 cd	27.0 ± 8.4 ab	73.0 ± 8.4 a	0.0 c
4.0	7.7 ± 1.8 a	1.8 ± 0.1 d	32.1 ± 8.1 a	67.9 ± 8.1 a	0.0 c

[z] Explants were cultured on B5 medium containing various concentrations of 6-benzylaminopurine (BA) and 0.1 mg L^{-1} α-naphthaleneaceticacid (NAA) for 8 wk. Nine explants were used in each treatment.

[y] For each variety, means in the same column followed by different letters are significantly different at the 5% level by least significant difference (LSD) test.

品種在葉片展開苗數與綠色芽數上皆隨著 BA 濃度提高而減少，但是淡黃色芽數則隨之增加；BA 濃度對於芽體型態之影響以「廣東薑」較「竹薑」明顯，前者在 BA ≥ 1 mg L^{-1} 處理下，淡黃色芽之比率皆超過 80% (表 2)。

液-固態兩階段培養對薑組培苗增殖與生長之影響

將上述「廣東薑」與「竹薑」於液態培養所得含葉片展開苗、綠色芽或淡黃色芽之叢生芽苗團塊，不經分割直接繼代培養於含 0.5 mg L^{-1} BA 與 0.1 mg L^{-1} NAA 之 B5 固態培養基 8 wk 後，多數芽體均可發育成為葉片展開苗 (圖 4)；成苗率則以「竹薑」較「廣東薑」高，前者在對照組與處理組均可達 100.0%，而後者以 4.0 mg L^{-1} BA 處理僅 55.6% 為最低，其餘 BA 處理組與對照組之成苗率則介於 66.7–88.9% 之間 (表 3)。表 3 結果顯示，源自 0.5–2.0 mg L^{-1} BA 處理之「廣東薑」可得 11.2–13.0 苗，與對照組所得 10.2 苗之間無顯著差異，苗高約為 2.8–3.1 cm，較對照組之 4.1 cm 低，而源自 4.0 mg L^{-1} BA 處理之苗數明顯低於其他 BA 處理組。「竹薑」結果顯示，源自 0.0–4.0 mg L^{-1} BA 處理之苗數間並無顯著差異，約在 11.2–14.9 苗之間，而源自 4.0 mg L^{-1} BA 處理之苗高雖顯著低於其他 BA 處理組，但仍可達約 4.2 cm (表 3)。

討論

薑組織培養所用之材料為根莖，因其位於地下而易附著塵土，並含有較多的細菌與真菌，導致初代培養的消毒成功率不佳。酒精、次氯酸鈉及氯化汞 (mercuric chloride; $HgCl_2$) 是較常用的消毒藥劑，部分學者也利用殺菌劑 (fungicide) 或抗生素 (antibiotic) 克服真菌或細菌汙染，但須搭配多種藥劑方能達成消毒效果 (Deng & Pan 2009; Kambaska & Santilata 2009; Kavyashree 2009; Abbas *et al*. 2011; Sathyagowri & Seran 2011; Seran 2013; David *et al*. 2016; Li 2016)。對於初代培養之效率而言，除上述消毒藥劑外，薑培植體大小亦為影響無菌培養成功與否的因子之一。雖然使用較

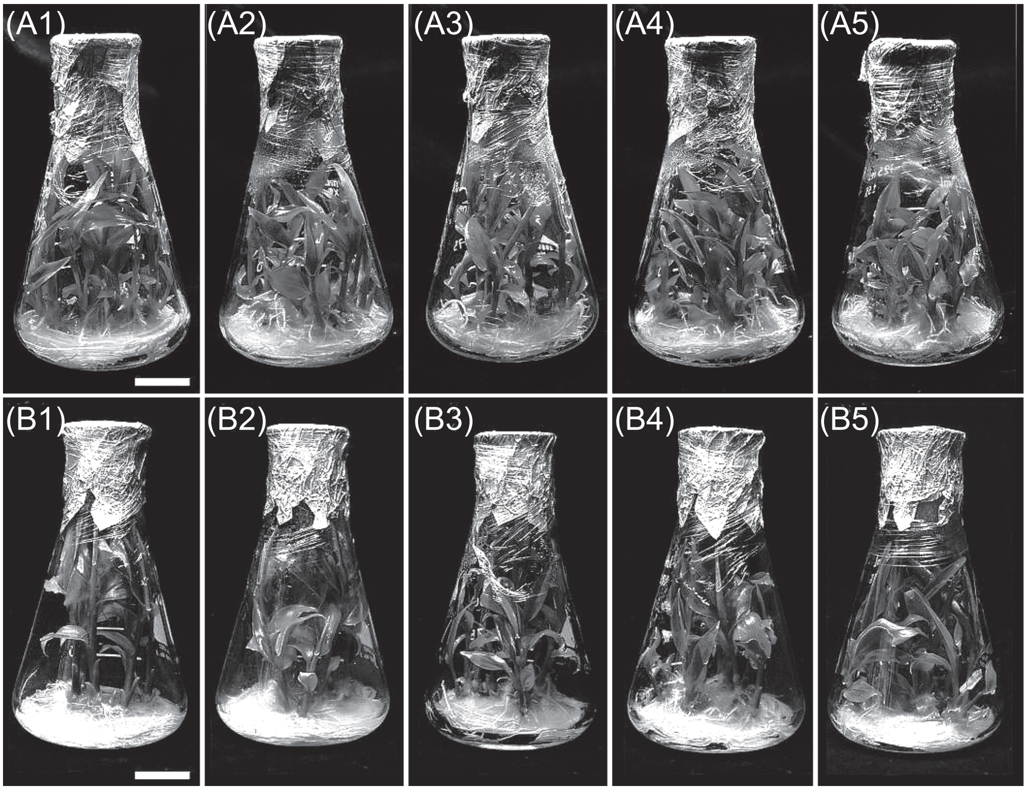

圖 2.　BA 濃度於固態培養下對「廣東薑」與「竹薑」組培苗增殖與生長之情形。「廣東薑」(A) 與「竹薑」(B) 之組培根莖分別培養於含有 0.0、0.5、1.0、2.0、4.0 mg L^{-1} BA (依序為 A 與 B 圖中之 1、2、3、4、5) 配合 0.1 mg L^{-1} α-naphthalene acetic acid (NAA) 之 B5 固態培養基 8 wk 後之瓶苗；Bar = 2 cm。

Fig. 2.　Effect of 6-benzylaminopurine (BA) concentration in the solid medium on plantlet proliferation and growth of *in vitro Zingiber officinale* 'Guang Dong' (A) and 'Chu' (B). Explants cultured on B5 solid medium supplemented with 0.0, 0.5, 1.0, 2.0, and 4.0 mg L^{-1} BA (1, 2, 3, 4, and 5 in A and B, respectively) and 0.1 mg L^{-1} α-naphthaleneacetic acid (NAA) for 8 wk of culture. Bar = 2 cm.

大培植體具有較佳之芽體誘導效果，但同時伴隨著污染率提高的問題 (Seran 2013)；一般而言，薑多以 5–10 mm 根莖芽體作為培植體 (Kavyashree 2009; Seran 2013)；而利用較小培植體 (0.3–0.5 mm)，則有避免病毒污染的優點 (Han 2000)。本研究以取自田間之「廣東薑」(圖 1) 與「竹薑」根莖經清潔後，誘導萌發新生根莖芽體為培植體，以酒精與次氯酸鈉溶液進行兩段式消毒的成功率約 70% (資料未列)，顯示在清潔環境下萌發之新生芽體不僅再生能力佳，且能提高後續之消毒成功率。

培養基中所含之植物生長調節劑對芽體的誘導與生長有直接影響，是影響薑組培苗繁殖效率的重要因子 (Seran 2013)。薑之莖頂與根莖芽體於不含 BA 之培養基時，並無法誘得芽體，但是在添加 BA 後即可促使芽體萌發，顯示 BA 為芽體誘導之必要條件 (Deng & Pan 2009; Kambaska & Santilata 2009; Kavyashree 2009)。Han (2000) 利用 0.3–0.5 mm「廣東薑」莖頂，培養於含有 2 mg L^{-1} BA 與 0.1 mg L^{-1} NAA 之 B5 培養基 90 d (約 3 mo) 後，每個莖頂可得約 5.5 個芽體。Huang *et al*. (2004) 將 0.4–0.5 mm「竹薑」生長點，培養於含有 2 mg L^{-1} BA 與 1 mg L^{-1} NAA 之 MS (Murashige & Skoog 1962) 培養基，增殖倍率為每月 3.5 芽。Ayenew *et al*. (2012) 以 15 mm 衣索比亞薑 (Ethiopian) 根莖芽體，培養於含有 2 mg L^{-1} BA 與 1 mg L^{-1} kinetin 之 MS 培養基 6 wk 後，

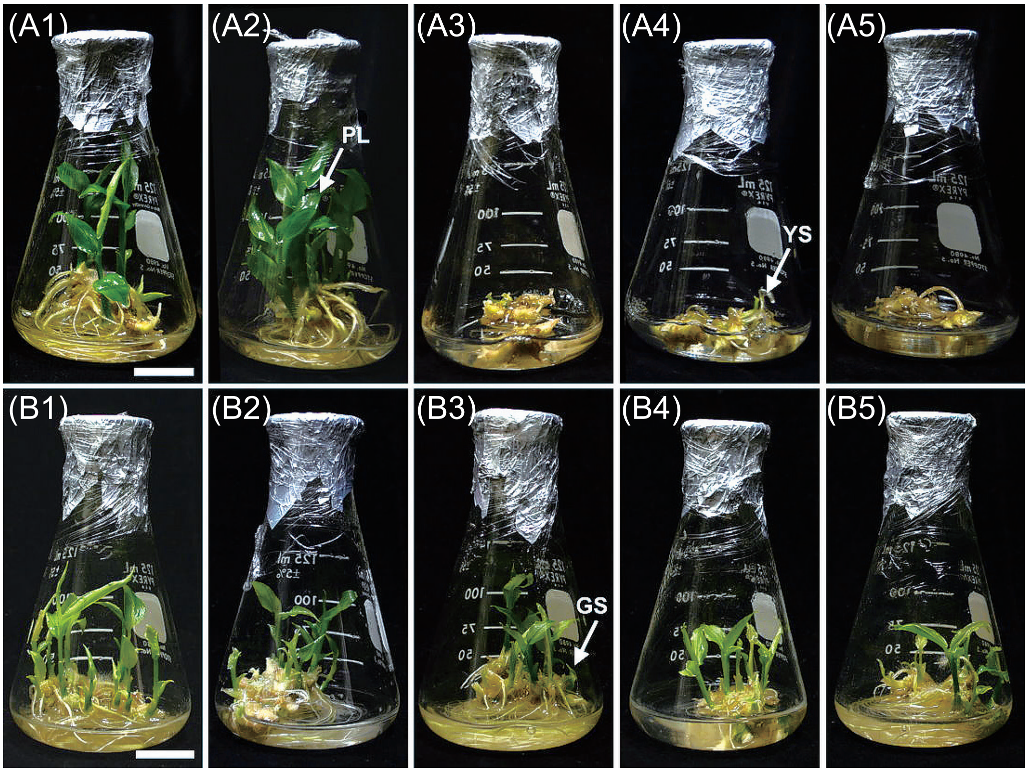

圖 3. BA 濃度於液態培養下對「廣東薑」與「竹薑」組培苗增殖與生長之情形。「廣東薑」(A) 與「竹薑」(B) 之組培根莖分別培養於含有 0.0、0.5、1.0、2.0、4.0 mg L^{-1} BA (依序為 A 與 B 圖中之 1、2、3、4、5) 配合 0.1 mg L^{-1} α-naphthalene acetic acid (NAA) 之 B5 液態培養基 8 wk 後所形成之葉片展開苗 (PL, A2)，以及葉片未開之綠色芽 (GS, B3) 與淡黃色芽 (YS, A4)；Bar = 2 cm。

Fig. 3. Effect of 6-benzylaminopurine (BA) concentration in the liquid medium on plantlet proliferation and growth of *in vitro Zingiber officinale* 'Guang Dong' (A) and 'Chu' (B). Plantlets with unfolded leaves (PL, A2), green shoot-bud (GS, B3), and yellowish shoot-bud (YS, A4) were obtained from the *in vitro* rhizome explant cultured on B5 liquid medium supplemented with 0.0, 0.5, 1.0, 2.0, and 4.0 mg L^{-1} BA (1, 2, 3, 4, and 5 in A and B, respectively) and 0.1 mg L^{-1} α-naphthaleneacetic acid (NAA) for 8 wk. Bar = 2 cm.

可形成約 7 株組培苗，且參試兩品種 ('Yali' 與 'Boziab') 有相類似的結果。埃及薑 (Egypt) 根莖芽體培養於含有 4.5 mg L^{-1} BA 之 MS 培養基 12 wk 後 (4 wk $time^{-1}$, 3 times)，可形成約 8 株組培苗 (Abbas *et al*. 2011)。Kavyashree (2009) 利用 5–10 mm 印度薑 ('Varada') 根莖芽體，培養於含有 17.76 μM (約 4 mg L^{-1}) BA 之 LS (Linsmaier & Skoog 1965) 培養基 4 wk 後，芽體增殖率達 4 倍。綜合上述研究結果顯示，生長點或根莖芽體培養於 2–4 mg L^{-1} BA 處理下，每 4 wk 之芽體增殖率分別為 1.8–3.5 芽與 2.7–4.7 芽，似乎培植體種類與大小對芽體增殖率並無明顯影響。Han (2000) 與 Huang *et al*. (2004) 分別以「廣東薑」或「竹薑」莖頂進行培養，每月芽體增殖率分別達 1.8 芽與 3.5 芽。本研究以「廣東薑」根莖芽體培養結果顯示，根莖芽體於含有 0.4 mg L^{-1} BA 之 B5 固態培養基 4 wk 後即可誘使生長。而後將此芽體繼代培養於相同組成之新鮮培養基 8 wk 後，僅獲得約 3–4 芽，亦即每 4 wk 之增殖率僅 1.0–1.1 芽 (資料未列，參考圖 1)，推測 BA 濃度對於薑芽體增殖率之高低具有明顯影響。此外，由於考量「廣東薑」與「竹薑」根莖芽體初代培養之高汙染率問題，因此先以較低 BA 濃度誘使根莖芽體生長後，再以較高 BA 濃度進行芽體增殖的培養策略。

表 2. BA 濃度於液態培養下對「廣東薑」與「竹薑」組培苗增殖與生長之影響。

Table 2. Effect of BA in the liquid medium on plantlet proliferation and growth of *in vitro Zingiber officinale* 'Guang Dong' and 'Chu'[z].

				Percent of shoot-bud (%)	
BA (mg L^{-1})	No. of plantlets	Plantlet height (cm)	Plantlet with unfolded leaves (%)	Green shoot-bud	Yellowish shoot-bud
'Guang Dong'					
0.0	6.1 ± 0.2 b[y]	1.4 ± 0.1 a	29.0 ± 11.3 a	32.8 ± 10.1 a	38.1 ± 4.4 c
0.5	7.8 ± 0.2 a	1.3 ± 0.2 a	25.4 ± 18.8 a	22.9 ± 6.8 a	51.7 ± 20.6 c
1.0	7.6 ± 0.5 a	1.3 ± 0.3 a	6.2 ± 4.7 ab	13.6 ± 9.8 a	80.3 ± 17.1 bc
2.0	7.6 ± 0.4 a	1.2 ± 0.0 a	5.9 ± 2.1 bc	7.4 ± 9.5 a	86.6 ± 12.2 ab
4.0	6.3 ± 0.9 b	-[x]	0.0 ± 9.5 c	4.9 ± 4.6 a	95.1 ± 4.6 a
'Chu'					
0.0	6.7 ± 1.0 a	1.6 ± 0.4 a	54.2 ± 7.7 a	23.7 ± 11.7 a	22.1 ± 4.3 c
0.5	7.1 ± 0.2 a	1.3 ± 0.1 a	48.8 ± 18.8 a	29.7 ± 1.9 a	21.6 ± 16.9 c
1.0	7.2 ± 0.6 a	1.3 ± 0.2 a	43.9 ± 4.7 ab	22.9 ± 6.5 a	33.2 ± 11.3 bc
2.0	7.3 ± 0.6 a	1.2 ± 0.1 a	27.4 ± 2.1 bc	24.7 ± 9.1 a	47.9 ± 8.0 ab
4.0	7.8 ± 1.3 a	1.3 ± 0.3 a	9.9 ± 9.5 c	24.6 ± 9.6 a	65.5 ± 14.0 a

[z] Explants were cultured on B5 medium containing various concentration of 6-benzylaminopurine (BA) and 0.1 mg L^{-1} α-naphthaleneacetic acid (NAA) for 8 wk. Nine explants were used in each treatment.

[y] For each variety, means in the same column followed by different letters are significantly different at the 5% level by least significant difference (LSD) test.

[x] No buds with unfolded leaves.

薑組培苗繁殖常利用 1–5 mg L^{-1} BA 配合 0.1–2 mg L^{-1} NAA 之 MS 培養基，此組成可誘導芽體形成而達到增殖效果，且 BA 配合 NAA 處理對芽體增殖效率顯著優於單獨使用 BA (Deng & Pan 2009; Kambaska & Santilata 2009; Seran 2013)。Li (2016) 將山東薑根莖芽體培養於含有 1.5 mg L^{-1} BA 與 0.5 mg L^{-1} NAA 之 MS 培養基 30 d 後，芽體增殖率達 3.5 倍。印度薑 'Suprava' 與 'Suruchi' 的研究結果顯示，長約 1 cm 根莖芽體培養於含有 2 mg L^{-1} BA 配合 0.5 mg L^{-1} NAA 之 MS 培養基 6 wk 後，誘得株高約 6.2 cm 之組培苗達 7.5 苗；若將 BA 濃度提高至 2.5–3 mg L^{-1}，則會形成癒傷組織且降低芽體數量 (Kambaska & Santilata 2009)。本研究結果顯示，提高 BA 濃度至 4.0 mg L^{-1} 雖可促進苗數增加，但苗高顯著降低；此外，本研究中使用 2.0–4.0 mg L^{-1} BA 濃度處理「廣東薑」與「竹薑」並未形成癒傷組織，亦未見芽數減少之現象。上述兩研究顯示不同的結果，推測其原因可能是品種間差異所造成。

Seran (2013) 之評論報告指出，薑組培芽體若培養於含有較高 BA 濃度之培養基中，則其伸長與發根均會受抑制，因此在芽體量化繁殖後，培養基需要調降 BA 濃度，誘使芽體伸長與發根後才能成為組培苗。Han (2000) 於「廣東薑」莖頂培養研究指出，其誘導所得之芽體並無法同時發根成苗，必須將芽體繼代培養於含有 1 mg L^{-1} NAA 之 MS 培養基中誘導發根。「竹薑」 (Huang *et al*. 2004) 與衣索比亞薑 (Ayenew *et al*. 2012) 利用生長點為培植體之研究結果亦顯示，芽體須經過伸長與發根階段才能成為組培苗。Sathyagowri & Seran (2011) 利用斯里蘭卡薑 'Local'、'Chinese' 及 'Ragoon' 的研究結果也顯示，組培苗的養成需要經過兩個階段，先將 5 mm 根莖芽體培養於含有 5 mg L^{-1} BA 與 0.5 mg L^{-1} NAA 之 MS 培養基 6 wk 後，誘導芽體成為叢生芽，而後再將叢生芽分切後，繼代培養於含有 3 mg L^{-1} BA 與 0.5 mg L^{-1} NAA 之 MS 培養基中，促進芽體伸長與發根而成為組培苗。印度薑 (Kambaska & Santilata 2009)、埃及薑 (Abbas *et al*.

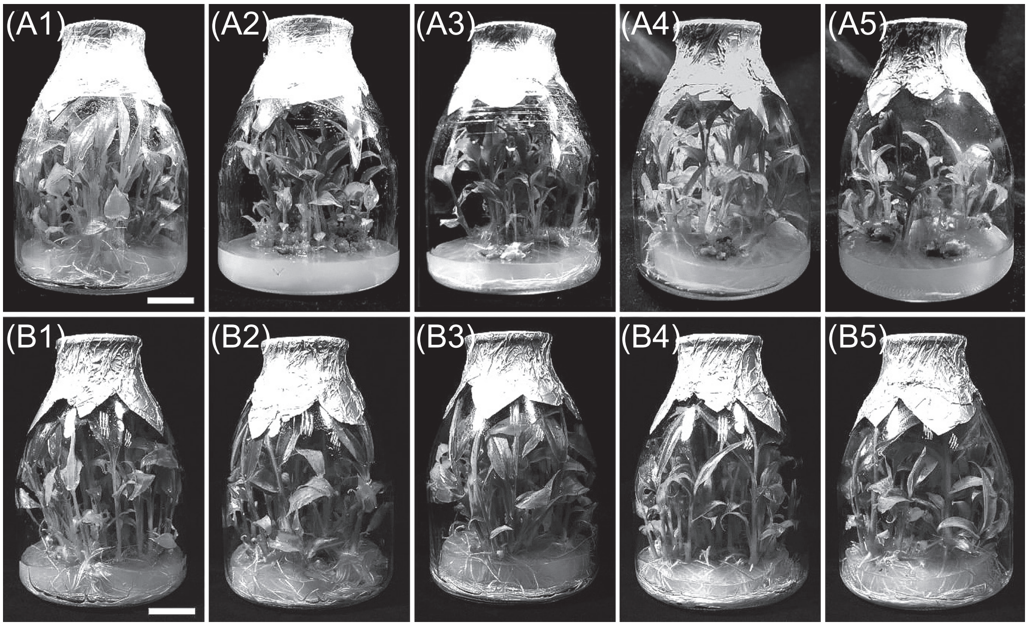

圖 4. 源自不同 BA 濃度液態培養之「廣東薑」與「竹薑」於液-固兩階段培養後組培苗增殖與生長之情形。「廣東薑」(A) 與「竹薑」(B) 之組培根莖先於含有 0.0、0.5、1.0、2.0、4.0 mg L^{-1} BA (依序為 A 與 B 圖中之 1、2、3、4、5) 配合 0.1 mg L^{-1} NAA 之 B5 液態培養基中培養 8 wk 後，將未經切割之葉片展開苗、綠色芽及淡黃色芽直接繼代培養於含有 0.5 mg L^{-1} BA 與 0.1 mg L^{-1} NAA 之 B5 固態培養基 8 wk 後之瓶苗生長情形；Bar = 1 cm。

Fig. 4. Effect of 6-benzylaminopurine (BA) and liquid-solid two-stage culture on plantlet proliferation and growth of *in vitro Zingiber officinale* 'Guang Dong' (A) and 'Chu' (B). Uncut explants were derived from B5 liquid medium containing 0.0, 0.5, 1.0, 2.0, and 4.0 mg L^{-1} BA (1, 2, 3, 4, and 5 in A and B, respectively) and 0.1 mg L^{-1} α-naphthaleneacetic acid (NAA) for 8 wk of culture before subculturing on the B5 solid medium containing 0.5 mg L^{-1} BA and 0.1 mg L^{-1} NAA for another 8 wk of culture. Bar = 1 cm.

2011) 及山東薑 (Li 2016) 均利用類似之組培苗量化繁殖策略，亦即先以較高 BA 濃度誘導大量芽體形成，而後再將其繼代培養於調降 BA 濃度之培養基中，則可促進芽體伸長與發根而成為正常瓶苗。

然而 Kavyashree (2009) 於印度薑 'Varada' 的研究指出，根莖芽體培養於含有 17.76 μM (4 mg L^{-1}) BA 之 LS 培養基 4 wk 後，可誘導芽體形成，再經過 2–3 次繼代於相同組成培養基，只須一種培養基組成即可發根成苗。本研究結果顯示，將根莖芽體培養於含有 0.4 mg L^{-1} BA 之 B5 培養基 2 次，共 12 wk 之培養後，即形成每苗含 3–4 芽且發根之組培苗 (圖 1C、1D)。由上述研究來看，不同之培植體與 BA 濃度會影響薑組培苗繁殖之效率；以生長點為培植體，須經芽體增殖與伸長發根兩個階段才能成為組培苗，而利用根莖芽體作為培植體，則其芽體較易發根而成苗，然而提高 BA 濃度則會抑制芽體伸長發根，必須將芽體繼代培養於調降 BA 濃度之培養基中，藉以促使芽體伸長與發根。推測根莖芽體基部可能是已分化之根莖組織，因此根系發育極為容易；然而，莖頂係尚未分化之地上部組織，因此根系發育則相對困難；而其他需要兩個階段才能成苗的相關研究，則可能是品種差異或 BA 濃度過高所導致的結果，抑或是組培苗量化繁殖的策略應用。

Seran (2013) 的評論指出，薑的芽體若培養於含有 1–5 mg L^{-1} BA 配合 0.0–0.5 mg L^{-1} NAA 之 MS 培養基，可同時增殖芽體與發根而成苗。David *et al*. (2016) 將 5–10 mm 馬來西亞薑 'Tambunan' 根莖芽體，培養於含有 3 mg L^{-1} BA 與 1 mg L^{-1} NAA 之 MS 培養基中 10 wk 後，可獲得 6.14 株發根組培苗。Lincy

表3. 源自不同BA濃度液態培養基之「廣東薑」與「竹薑」培植體於固態培養基中對組培苗增殖與生長之影響。

Table 3. Effect of explants derived from various BA concentrations in the liquid medium on plantlet proliferation and growth after subculturing in the solid medium of *Zingiber officinale* 'Guang Dong' and 'Chu'[z].

BA (mg L^{-1})	No. of plantlets	Plantlet height (cm)	Plantlet formation (%)
'Guang Dong'			
0.0	10.2 ± 1.4 ab[y]	4.1 ± 0.6 a	88.9 ± 19.2 a
0.5	12.5 ± 2.9 a	2.8 ± 0.3 b	88.9 ± 19.2 a
1.0	13.0 ± 4.5 a	3.1 ± 0.3 b	66.7 ± 0.0 ab
2.0	11.2 ± 1.5 a	2.8 ± 0.4 b	88.9 ± 19.2 a
4.0	3.9 ± 5.3 b	2.4 ± 0.8 b	55.6 ± 19.2 b
'Chu'			
0.0	11.2 ± 1.2 a	6.1 ± 0.6 a	100.0 a
0.5	14.9 ± 3.3 a	5.2 ± 0.9 ab	100.0 a
1.0	13.5 ± 1.3 a	5.0 ± 0.3 bc	100.0 a
2.0	13.3 ± 2.5 a	4.6 ± 0.2 bc	100.0 a
4.0	14.9 ± 2.0 a	4.2 ± 0.3 c	100.0 a

[z] Explants derived from B5 liquid medium containing 0–4 mg L^{-1} 6-benzylaminopurine (BA) and 0.1 mg L^{-1} α-naphthaleneacetic acid (NAA) for 8 wk of culture, and the explants without cutting were subcultured on the B5 solid medium supplemented with 0.5 mg L^{-1} BA and 0.1 mg L^{-1} NAA for another 8 wk of culture. Nine explants were used in each treatment.

[y] For each variety, means in the same column followed by different letters are significantly different at the 5% level by least significant difference (LSD) test.

& Sasikumar (2010) 將 1.0–1.5 cm 印度薑根莖芽體，培養於含有 1.0 mg L^{-1} 賽本隆 (thidiazuron, TDZ) 配合 0.5 與 1.0 mg L^{-1} 吲哚丁酸 (indole butyric acid; IBA) 之 MS 培養基中 2 mo 後，兩品種 'Jamaica' 與 'Varada' 分別達 8.8–12.9 個芽與 6.8–8.1 條根。本研究結果顯示，0.5–2.0 mg L^{-1} BA 配合 0.1 mg L^{-1} NAA 處理之每個培植體可誘得約 5–6 苗，苗高約 1.8–2.6 cm (表 1)；提高 BA 濃度至 4.0 mg L^{-1} 雖可增加苗數，但苗高顯著較低；反之，對照組之苗高雖然較高，但苗數卻顯著較少 (表 1)；此外，在此增殖階段之組培芽體均可同時發根良好而成苗 (圖 2)，因此本研究並未調查各 BA 處理所得組培苗之根數與根長。綜合上述研究結果指出，薑根莖培植體培養於含有適當細胞分裂素 (BA 與 TDZ) 與生長素 (NAA 與 IBA) 濃度組合之處理，則可具有促進芽體增殖、伸長及發根的效果。本研究於「廣東薑」與「竹薑」組培苗數量與高度之結果顯示，雖然對照組之組培苗顯著較高，且芽數上與 0.5 mg L^{-1} BA 處理組並無顯著差異，但是 BA 處理具有較多芽數，考量為維持後續培養時之增殖、伸長及發根效率，建議可利用 0.5 mg L^{-1} BA 配合 0.1 mg L^{-1} NAA 之 B5 培養基，作為薑組培苗之繼代培養基。

Luo *et al*. (2006) 利用 30 mL 固態培養基或於固態培養基上添加少量 10 mL 液態培養基的淺層液態培養方式，進行「廣東薑」組培苗繁殖研究，結果顯示固態與淺層液態培養方式在培養 1 mo 後之發根率均可達 100%，但是淺層液態處理之組培苗葉片較大且植株較為健壯。Lincy & Sasikumar (2010) 利用 1.0–1.5 cm 印度薑 'Jamaica' 與 'Varada' 地上部芽體，培養於只含有 TDZ 的固態培養基，所誘得的芽體數較少且大多發育異常，若以液態培養則可顯著提高芽體增殖率；而後將叢生芽體培養於含有 1 mg L^{-1} TDZ 配合 0.5 或 1.0 mg L^{-1} IBA 之 MS 液態培養基中 3 wk 後，芽體伸長且發根成為白色叢生苗；而後再將每叢含有 4–5 芽之叢生苗作為培植體，培養於含有 1 mg L^{-1} BA 與 1 mg L^{-1} NAA 之 MS 固態培養基中約 45 d 後，即可形成發根良好的綠色叢生組培苗。雖然報告中並未提及芽體增殖率，但可知液 - 固兩階段培養方式具有促進組培苗增殖與

生長效果。本研究於「廣東薑」與「竹薑」也顯示相類似結果，雖然液態培養下每個培植體獲得約 6.1–7.8 芽，但卻存在大量小於 1 cm 的淡黃色芽 (表 2、圖 3)。因此，後續再將叢生芽繼代培養於固態培養基中，以促使大量芽體伸長且發根成為正常苗。表 3 與圖 4 之結果顯示，「廣東薑」與「竹薑」於液態培養下所得叢生芽團塊，不經切割而直接繼代培養於固態培養基中，可使「廣東薑」芽苗團塊中部分芽體長成正常苗，其成苗率約 55.6–88.9%，而「竹薑」之成苗率更可達到 100%；就瓶苗增殖率而言，兩品種均以源自 0.5–2.0 mg L^{-1} BA 液態處理之結果較佳，顯示液-固態兩階段培養可利用於薑組培苗之大量繁殖。此外，「廣東薑」與「竹薑」經液態與固態兩階段共 16 wk 培養後，每 8 wk 的平均增殖率分別為 6.50 (13 苗/2 次) 與 7.45 (14.9 苗/2 次) (表 3)；相較於固態培養 8 wk 後所得 7.2 與 7.7 之增殖率的差異不大，但兩階段培養方式之優點在於所得瓶苗較高 (表 1、表 3)，且在液態培養後繼代培養時，無須切割直接轉入固態培養基中。而固態培養則因根系已發育完成，繼代培養時必須切除部分假莖與根系，兩培養方式在操作程序比較下，液-固態兩階段培養方式顯然較為省工，但是卻也存在液態培養所需振盪培養設備需求的問題。

本研究利用「廣東薑」與「竹薑」之根莖芽體，已建立其微體繁殖方式，可將組培芽體培養於含有 0.5 mg L^{-1} BA 與 0.1 mg L^{-1} NAA 之 B5 固態培養基，可達瓶苗增殖、伸長及發根成苗之目的。此外，本研究同時建立液-固態兩階段養方式，不僅有效促進組培苗之增殖率，且可縮短組培操作之時間；後續將針對苗數與苗高對於薑苗生長的重要性，並探討組培苗之適當馴化條件，完成量產優質薑種苗之生產鏈，提供薑種苗產業之應用。

誌謝

本文稿承農業試驗所花卉研究中心蔡媚婷博士協助審閱，特此申謝。

引用文獻

Abbas, M. S., H. S. Taha, U. I. Aly, H. M. El-Shabrawi, and E. I. Gaber. 2011. *In vitro* propagation of ginger (*Zingiber officinale* Rosco). J. Gene. Engine. Biotech. 9:165–172.

Agrahari, P., P. Panda, N. K. Verma, W. U. Khan, and S. Darbari. 2015. A brief study on *Zingiber officinale*-A review. J. Drug Dis. Therap. 3:20–27.

Ali, B. H., G. Blunden, M. O. Tanira, and A. Nemmar. 2008. Some phytochemical, pharmacological and toxicological properties of ginger (*Zingiber officinale* Roscoe): A review of recent research. Food Chem. Toxicol. 46:409–420.

Ayenew, B., W. Tefera, and B. Kassahun. 2012. *In vitro* propagation of Ethiopian ginger (*Zingiber officinale* Rosc.) cultivars: Evaluation of explant types and hormone combinations. Afr. J. Biotechnol. 11:3911–3918.

Chen, W. L., R. S. Li, Z. H. Tsai, and C. H. Hsiao. 2014. Study on improving sprout forcing methods for early ginger (*Zingiber officinale*) production. J. Taiwan Soc. Hort. Sci. 60:253–264. (in Chinese with English abstract)

Council of Agriculture, Executive Yuan. 2017a. A survey of vegetable production in Taiwan. http://agrstat.coa.gov.tw/sdweb/public/official/OfficialInformation.aspx (visit on 2017/6/6) (in Chinese)

Council of Agriculture, Executive Yuan. 2017b. Yearly report of Taiwan's agriculture. http://agrstat.coa.gov.tw/sdweb/public/book/Book.aspx (visit on 2017/6/6) (in Chinese)

David, D., T. Y. Ji, and J. A. Gansau. 2016. *In vitro* propagation of *Zingiber officinale* Rosc. 'Tambunan'. Trans. Sci. Technol. 3:162–167.

Deng, N. F. and B. L. Pan. 2009. Research progress on tissue culture of ginger. J. Anhui Agric. Sci. 37:12406–12407, 12410. (in Chinese with English abstract)

Food and Agriculture Organization of the United Nations. 2017. Ginger visualize data in Food and Agricultural Organization of the United Nations. http://www.fao.org/faostat/en/#data/QC/visualize (visit on 2017/6/6)

Gamborg, O. L., R. A. Miller, and O. Ojima. 1968. Nutrient requirements of suspension cultures of soybean root cells. Exp. Cell Res. 50:151–158.

Han, C. M. 2000. Study on the shoot tip culture of ginger (*Zingiber officinale* Roscoe). Res. Bull. KDARES 11:37–46. (in Chinese with English abstract)

Hsieh, L. Y., C. J. Tsai, C. Y. Huang, and H. I. Chen. 2017. Production forecast for May 2017. http://

www.coa.gov.tw/ws.php?id=2506392 (visit on 2017/6/6) (in Chinese)

Huang, Y. X., J. C. Wang, F. F. Hem. D. B. Tang, C. Cui, C. H. Li, and Z. W. Liu. 2004. Studies of technology systems for meristem culture and rapid propagation of ginger (*Zingiber officinals*). J. Southwest Agric. Univ. (Nat. Sci.) 26:556–559. (in Chinese with English abstract)

Kambaska, K. B. and S. Santilata. 2009. Effect of plant growth regulator on micropropagation of ginger (*Zingiber officinale* Rosc.) cv- Suprava and Suruchi. J. Agric. Technol. 5:271–280.

Kavyashree, R. 2009. An efficient *in vitro* protocol for clonal multiplication of Ginger-var. Varada. Indian J. Biotechnol. 8:328–331.

Li, F. 2016. Study on tissue culture and transplanting techniques of ginger. J. Liaoning Agric. Tech. College 18:13–14, 20. (in Chinese with English abstract)

Lincy, A. and B. Sasikumar. 2010. Enhanced adventitious shoot regeneration from aerial stem explants of ginger using TDZ and its histological studies. Turk. J. Bot. 34:21–29.

Linsmaier, E. M. and F. Skoog. 1965. Organic growth factor requirements of tobacco tissue cultures. Physiol. Plant. 18:100–127.

Luo, T. K., X. L. Zhang, Z. Chen, Z. Tang, Q. Liu, and S. J. Zhu. 2006. Application of shallow liquid culture system for the rapid propagation technology of virus-free ginger. Chinese Agric. Sci. Bull. 22:75–77. (in Chinese with English abstract)

Mishra, R. K., A. Kumar, and A. Kumar. 2012. Pharmacological activity of *Zingiber officinale*. Intl. J. Pharm. Chem. Sci. 1:1073–1078.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant. 15:473–497.

Rehman, R., M. Akram, N. Akhtar, Q. Jabeen, T. Saeed, S. M. Ali Shah, K. Ahmed, G. Shaheen, and H. M. Asif. 2011. *Zingiber officinale* Roscoe (pharmacological activity). J. Med. Plants Res. 5:344–348.

Sathyagowri, S. and T. H. Seran. 2011. *In vitro* plant regeneration of ginger (*Zingiber officinale* Rosc.) with emphasis on initial culture establishment. Intl. J. Med. Arom. Plants 1:195–202.

Seran, T. H. 2013. *In vitro* propagation of ginger (*Zingiber officinale* Rosc.) through direct organogenesis: A review. Pak. J. Biol. Sci. 16:1826–1835.

Singh, S. K., J. R. Patel, and D. Bachle. 2014. A review on *Zingiber officinale*: A natural gift. Intl. J. Pharm. Bio. Sci. 5:508–525.

Tsai, J. H., H. L. Liu, C. W. Chen, Y. F. Tsai, and C. S. Chang. 2013. Studies on continuous cropping disease of ginger and its shoot promoting technology. Seed Nursery (Taiwan) 15:57–67. (in Chinese with English abstract)

Zadeh, J. B. and N. M. Kor. 2014. Physiological and pharmaceutical effects of ginger (*Zingiber officinale* Roscoe) as a valuable medicinal plant. Euro. J. Exp. Bio. 4:87–90.

Effect of Plant Growth Regulator and Two-Stage Culture on Proliferation and Growth of *In Vitro* Ginger Plantlets

Uei-Chern Chen[1], Chin-Yi Tsao[1], Tzu-Ying Wu[2], and Chi-Ni Hsia[3,*]

Abstract

Chen, U. C., C. Y. Tsao, T. Y. Wu, and C. N. Hsia. 2018. Effect of plant growth regulator and two-stage culture on proliferation and growth of *in vitro* ginger plantlets. J. Taiwan Agric. Res. 67(4):401–413.

Efficient protocols for *in vitro* multiplication of *Zingiber officinale* 'Guang Dong' and 'Chu' were established in this study. Newly germinated rhizome buds (about 1 cm) were treated with 75% alcohol for 30 s, followed by 0.6% NaOCl for 15 min, before cultured on a B5 basal medium containing 0.4 mg L^{-1} 6-benzyladeninepurine (BA) for 12 wk to establish *in vitro* culture. Proliferation of 4.8–6.0 plantlets per explant with 1.8–2.6 cm in height were obtained by using 5 mm rhizome explants culturing on a B5 solid medium containing 0.5–2.0 mg L^{-1} BA and 0.1 mg L^{-1} α-naphthalene acetic acid (NAA) for 'Guang Dong' and 'Chu'. Good rooting was found for all plantlets derived from all treatments. Although more plantlets were obtained in the 4.0 mg L^{-1} BA treatment, shorter plantlets were observed. On the contrary, higher plantlets with less numbers were found from the control. Proliferation of 7.6–7.8 and 7.1–7.3 plantlets per explant were obtained for 'Guang Dong' and 'Chu', respectively, by using 5 mm rhizome explants culturing on the liquid medium containing 0.5–2.0 mg L^{-1} BA for 8 wk. However, plantlets with unfolded leaves of 1.2–1.3 cm in height were observed for both varieties. Uncut explants of plantlets with unfolded leaves, green shoot-buds, and yellowish shoot-buds derived from the liquid culture were subcultured onto the fresh solid B5 medium containing 0.5 mg L^{-1} BA and 0.1 mg L^{-1} NAA for another 8 wk. Proliferation of 11.2–13.0 plantlets per explant with 2.8–3.1 cm in height and 13.3–14.9 plantlets per explant with 4.6–5.2 cm in height were obtained for 'Guang Dong' and 'Chu', respectively. This result indicated that using liquid-solid two-stage culture could be beneficial on *in vitro* multiplication of *Z. officinale*.

Key words: *Zingiber officinale*, Liquid-solid culture, 6-benzylaminopurine, Mass propagation.

Received: September 29, 2017; Accepted: June 21, 2018.
[*] Corresponding author, e-mail: hsia@tari.gov.tw
[1] Assistant Research Fellows, Biotechnology Division, Taiwan Agricultural Research Institute, Taichung, Taiwan, ROC.
[2] Research Assistant, Biotechnology Division, Taiwan Agricultural Research Institute, Taichung, Taiwan, ROC.
[3] Research Fellow, Biotechnology Division, Taiwan Agricultural Research Institute, Taichung, Taiwan, ROC.

台 灣 農 業 研 究

(原《中華農業研究》期刊)

第六十七卷　目次

第一期

專題論述

高景輝	水稻鎘逆境：矽元素的影響	1

研究報告

鄭允、王志賢、黃毓斌、江明耀、呂秀英、吳東鴻、楊崇民、粘君琪	瓜實蠅 [*Bactrocera* (*Zeugodacus*) *cucurbitae* (Coquillett)] 中胸背板剛毛數量性狀之遺傳交互作用	9
蔡佳欣、安寶貞、呂昀陞、黃淑苓、洪挺軒	*Pseudomonas mediterranea* 引起之番茄髓壞疽病	16
廖大經、郭介煒、郭素真、連怡鈞、林彥蓉、吳永培	利用分子標幟輔助水稻新品系堆疊耐旱與耐鹽性	28
陳威臣、吳姿穎、曹進義、夏奇鈮	兩階段培養及巴克素對孤挺花組培苗生長與發根之影響	44
廖大經、吳佩真、吳東鴻	針對稻熱病 *Pia* 及 *Piz* 基因座分子輔助選育秈稻新品系之研究	54
黃哲倫、徐敏記、羅淑芳、賴永昌	抑芽劑 CIPC、鈷 60 輻射照射對甘藷貯藏後發芽等性狀之影響	73
周思儀、廖大經	2009 至 2014 年台灣水稻新育成品種 (系) 對於稻熱病罹病反應之研究	82
夏奇鈮、曹進義、吳姿穎、陳威臣	四倍體丹參毛狀根系之時序培養及其丹參酮生產篩選	94

第二期

研究報告

李奇峯、Lukáš Sekerka	金門產新記錄金花蟲：黃腹小扁鐵甲蟲的重新描述 (鞘翅目：金花蟲科：龜金花蟲亞科：小扁鐵甲蟲族)	107

Serguei V. Triapitsyn	台灣纓小蜂科 (膜翅目：小蜂總科) 名錄記述暨五新種描述	113
陳柏宏、王泰權、陳祈男、黃瓊慧、黃守宏	柑橘銹蟎 [*Phyllocoptruta oleivora* (Ashmead)] 在三種柑橘上之族群動態與空間分布	166
賴信順、陳怡如、邱一中、林鳳琪	溫度依變下小黃薊馬 (*Scirtothrips dorsalis* Hood) 在檬果上之發育及生命表	181
周建銘、陳君弢、簡伊萱、蔡錦慧、陳金枝、林鳳琪、陳怡如、鄧汀欽	玉米褪綠斑駁病毒 (*Maize chlorotic mottle virus*) 之親緣分析與台灣之發生調查	191
關政平、陳柏亨、陳涵葳、馮騰永、楊佐琦	基因轉殖PFLP香蕉於促進抗香蕉黃葉病之研究	209

研究簡報

何琦琛、王順成、梁世祥、張淑貞	長莖全爪蟎 (*Panonychus elongatus* Manson) (絨蟎目：葉蟎科)—一種新興的害蟎	218
董耀仁、許北辰	己酸緩和棉蚜 (*Aphis gossypii* Glover) 對小黃瓜侵襲	224

第三期

研究報告

陳金枝、江芬蘭、黃春惠	宮燈百合 *Gloriosa stripe mosaic virus* 之核酸分子和免疫法鑑定	229
曹進義、陳威臣、吳容儀、吳姿穎、夏奇鈮	秋水仙素處理對藍紫色蝴蝶蘭 *Phalaenopsis pulcherrima* fma. *coerulea* 原球體多倍體誘導之影響	247
李香誼、李碩朋、許淼淼、羅惠齡	營農型光電溫室環境對多種葉菜類蔬菜生長之影響	258
林玫珠、林羿廷、蔡錦慧、鄧汀欽	分蔥潛隱病毒親緣分析及應用巢式聚合酶鏈鎖反應作分群偵測	270
董耀仁、許北辰	評估水楊酸甲酯對捕食性天敵的誘引效果	283
謝靜敏、楊正釧、盧虎生、莊曜駿、徐光平、李雅琳	油茶籽儲藏條件對壓榨油品質之影響	292

研究簡報

周建銘、陳君弢、蔡錦慧、陳金枝、鄧汀欽	以含 ToMV、ORSV 及 CGMMV 表位基因的合成聚核苷酸所表現的蛋白製備Tobamovirus廣效性抗體的抗原性	301

黃晉興、蘇俊峰、陳泊菘	利用夜間照光與割除枝葉以調控茭白筍採收期	309
蔡志濃、安寶貞、林筑蘋、蔡惠玲	靈芝引起的檳榔基腐病	318

第四期

研究報告

王清玲、陳怡如、林鳳琪	台灣寬薊馬亞科 (纓翅目：管尾亞目：管尾薊馬科) 之分類描述 I 雙毛族	323
鄭允、王志賢、黃毓斌、江明耀、呂秀英、吳東鴻、楊崇民、粘君琪	瓜實蠅 [*Bactrocera* (*Zeugodacus*) *cucurbitae* (Coquillett)] 中胸背板剛毛遺傳變異之縮減	337
曹麗玉	番椒微斑病毒 (*Pepper mild mottle virus*) 田間發生與器械消毒管理	347
楊淑惠、楊惠婷、蔣淑琦	香菇柄水萃物改善糖尿病降低阿茲海默氏症罹病風險初探	355
許健輝、林毓雯、鄭佳怡、何雅婷、陳柱中、劉滄棽、郭鴻裕	評估氯化鐵清洗法對含鎘水稻田土壤鎘移除、土壤性質及稻作生長之影響	365
林靜宜、林慧如	利用中和亞磷酸溶液防治馬鈴薯青枯病	377
黃巧雯、吳昭蓉、楊宏仁、賴素玉、倪蕙芳	柑橘褐斑病之病原特性及防治藥劑篩選	387
陳威臣、曹進義、吳姿穎、夏奇鋸	植物生長調節劑與兩階段培養對薑組織培養苗增殖與生長之影響	401

JOURNAL OF TAIWAN AGRICULTURAL RESEARCH

(Formerly Journal of Agricultural Research of China)

Contents of Volume 67

Number 1

Review Article

Ching Huei Kao	Cadmium Stress in Rice: Influence of Silicon	1

Research Articles

Edward Yun Cheng, Zu-Hsien Wang, Yu-Bing Huang, Ming-Yaw Chiang, Hsiu-Ying Lu, Dong-Hong Wu, Chung-Ming Yang, and Chun-Chi Nien	The Genetic Interaction on the Quantitative Traits of Prescutellar Bristles in Melon Fly, *Bactrccera* (*Zeugodacus*) *cucurbitae* (Coquillett)	9
Chia-Hsin Tsai, Pao-Jen Ann, Yun-Sheng Lu, Shu-Ling Hwang, and Ting-Hsuan Hung	Occurrence of Pith Necrosis of Tomato Caused by *Pseudomonas mediterranea* in Taiwan	16
Dah-Jing Liao, Chieh-Wei Kuo, Su-Chen Kuo, I-Chun Lien, Yann-Rong Lin, and Yong-Pei Wu	The Pyramiding of Drought and Salt Tolerance of New Rice Lines Using Marker-Assisted Selection	28
Uei-Chern Chen, Tzu-Ying Wu, Jhin-Yi Tsao, and Chi-Ni Hsia	Effects of Two-Stage Culture and Paclobutrazol on *In Vitro* Bulblet Growth and Rooting of *Hippeastrum hybridum*	44
Dah-Jing Liao, Pei-Chen Wu, and Dong-Hong Wu	Study on Development of Indica Breeding Lines Introgressed Blast Gene for *Pia* and *Piz* Loci through Marker-Assisted Backcross Approach	54
Che-Lun Huang, Min-QI Xu, Shu-Fang Lo, and Yung-Chang Lai	Effects of Treatments of Sprouting Inhibitor CIPC and Co60 on Sprouting and Decaying Rates of the Storage Roots of Sweet Potato	73
Szu-Yi Chou and Dah-Jing Liao	Field Evaluation of Blast for Rice Varieties (Lines) Developed from 2009 to 2014 in Taiwan	82
Chi-Ni Hsia, Chin-Yi Tsao, Tzu-Ying Wu, and Uei-Chern Chen	Screening on Tanshinone Production and Time Course Culture of Tetraploidy Hairy Roots of *Salvia miltiorrhiza*	94

Number 2

Review Articles

Chi-Feng Lee and Lukáš Sekerka	Redescription of *Leptispa abdominalis* Baly, 1858 (Insecta: Coleoptera: Chrysomelidae: Cassidinae: Leptispini), Newly Recorded from Kinmen Island, with Notes on Its Biology	107
Serguei V. Triapitsyn	An Annotated Checklist of Mymaridae (Hymenoptera: Chalcidoidea) in Taiwan, with Descriptions of Five New Species	113
Po-Hung Chen, Tai-Chuan Wang, Chi-Nan Chen, Chiung-Hui Huang, and Shou-Horng Huang	Population Dynamic and Spatial Distribution of the Citrus Rust Mite, *Phyllocoptruta oleivora* (Ashmead), on Three Varieties of Citrus in Taiwan	166
Hsin-Shun Lai, Yi-Ju Chen, Yi-Chung Chiu, and Feng-Chyi Lin	Temperature-dependent Development and Life Table of *Scirtothrips dorsalis* Hood (Thysanoptera: Thripidae) on Mango	181

Chien-Ming Chou, Chun-Tao Chen, Yi-Hsuan Chien, Chin-Hui Tsai, Chin-Chih Chen, Feng-Chyi Lin, Yi-Ju Chen, and Ting-Ching Deng	Phylogenetic Analysis and Occurrence of *Maize chlorotic mottle virus* Infecting Maize in Taiwan	191
Cheng-Ping Kuan, Po-Heng Chen, Han-Wei Chen, Teng-Yung Feng, and Tso-Chi Yang	Improving Resistance to *Fusarium* Wilt Disease in PFLP Transgenic Bananas	209

Short Communications

Chyi-Chen Ho, Shun-Cheng Wang, Shih-Hsiang Liang, and Shu-Chen Chang	*Panonychus elongatus* (Trombidiformes: Tetranychidae), a New Prosperous Crop Pest in Taiwan	218
Yaw-Jen Dong, and Bei-Chen Hsiu	Hexanoic Acid Alleviated Cotton Aphid (*Aphis gossypii* Glover) Infestations in Cucumber	224

Number 3

Research Articles

Chin-Chih Chen, Fen-Lang Chiang, and Chun-Huey Huang	Molecular and Serological Identification of *Gloriosa stripe mosaic virus* on Christmas Bells (*Sandersonia aurantiaca* Hook)	229
Chin-Yi Tsao, Uei-Chern Chen, Rung-Yi Wu, Tzu-Ying Wu, and Chi-Ni Hsia	Effects of Colchicine Treatments on Polyploidy Induction Using Protocorms of *Phalaenopsis pulcherrima* fma. *coerulea*	247
Hsiang-I Lee, Shough-Peng Lee, Miao-Miao Hsu, and Hui-Ling Lo	Growth Evaluation on Different Vegetable Species Grown in a Photovoltaic Greenhouse with 40% Coverage of Solar Panel on the Roof	258
Mei-Ju Lin, Yi-Ting Lin, Chin-Hui Tsai, and Ting-Chin Deng	Phylogenetic Analysis and Grouping Detection of Species *Shallot latent virus* by Nested-PCR	270
Yaw-Jen Dong and Bei-Chen Hsiu	Evaluating the Effectiveness of Methyl Salicylate on Attracting Predators	283
Ching-Ming Hsieh, Jeng-Chuann Yang, Huu-Sheng Lur, Yao-Chun Chuang, Kuang-Ping Hsu, and Ya-Lin Lee	Influence of Storage Conditions of *Camellia brevistyla* Seeds on the Quality of Pressed Oils	292

Short Communications

Chien-Ming Chou, Chun-Tao Chen, Chin-Hui Tsai, Chin-Chih Chen, and Ting-Chin Deng	Immunogenicity of Tobamovirus Universal Antibodies Raised Against Expressed Protein Encoded by a Synthetic Polynucleotide Containing Epitope Genes of ToMV, ORSV, and CGMMV	301
Jin-Hsing Huang, Jiunn-Feng Su, and Po-Sung Chen	Regulating the Harvest Period of Water Bamboo by Use of Night-time Lighting and Shoots Cut-off	309
Jyh-Nong Tsai, Pao-Jen Ann, Chu-Ping Lin, and Hui-Ling Tsai	Basal Stem Rot of Betel Palm Caused by *Ganoderma boninense* in Taiwan	318

Number 4

Research Articles

Chin-Ling Wang, Yi-Ju Chen, and Feng-Chyi Lin	Taxonomic Description of Idolothripinae (Thysanoptera: Tubulifera: Phlaeothripidae) of Taiwan. I. Tribe Idolothripini	323
Edward Yun Cheng, Zu-Hsien Wang, Yu-Bing Huang, Ming-Yaw Chiang, Hsiu-Ying Lu, Dong-Hong Wu, Chung-Min Yang, and Chun-Chi Nien	Reduction in Genetic Variability for Quantitative Trait Prescutellar Bristle Number of Melon Fly [*Bactrocera* (*Zeugodacus*) *cucurbitae* (Coquillett)]	337

Li-Yu Tsao	Investigation of *Pepper mild mottle virus* on Peppers and the Disinfection of Cutting Tools in Taiwan	347
Shu-Hui Yang, Hui-Ting Yang, and Shu-Chi Chiang	Preliminary Study of *Lentinus edodes* Stipe Extracts on Reducing the Risk of Alzheimer's Disease in Diabetic Animal Model	355
Chien-Hui Syu, Yu-Wen Lin, Chia-Yi Cheng, Ya-Ting Ho, Chu-Chung Chen, Tsang-Sen Liu, and Horng-Yuh Guo	Assessing the Effects of Soil Washing with Ferric Chloride on Cadmium Removal, Soil Properties and Rice Growth in Cd-containing Paddy Fields	365
Ching-Yi Lin and Hui-Ju Lin	Control of Bacterial Wilt of Potato with Neutralized Phosphorous Acid	377
Chiao-Wen Huang, Chao-Jung Wu, Hong-Ren Yang, Su-Yu Lai, and Hui-Fang Ni	Physiological Characteristics, Pathogenicity and Fungicide Screening of Citrus Alternaria Brown Spot Disease Caused by *Alternaria alternata*	387
Uei-Chern Chen, Chin-Yi Tsao, Tzu-Ying Wu, and Chi-Ni Hsia	Effect of Plant Growth Regulator and Two-Stage Culture on Proliferation and Growth of *In Vitro* Ginger Plantlets	401

台 灣 農 業 研 究

作者索引

Lukáš Sekerka	112
Serguei V. Triapitsyn	165
王志賢	15, 346
王泰權	166
王清玲	336
王順成	218
安寶貞	16, 318
江明耀	9, 346
江芬蘭	229
何琦琛	218
吳永培	28
吳佩真	54
吳東鴻	15, 54, 346
吳昭蓉	387
吳姿穎	44, 94, 247, 401
吳容儀	247
呂秀英	15, 346
呂昀陞	16
李奇峯	112
李香誼	258
李雅琳	292
李碩朋	258
何雅婷	365
周建銘	191, 308
周思儀	82
林玫珠	270
林彥蓉	28
林羿廷	270
林筑蘋	318
林毓雯	365
林鳳琪	181, 191, 336
林慧如	377
林靜宜	377
邱一中	181
洪挺軒	16
夏奇鈮	44, 94, 247, 401
徐光平	292
徐敏記	73
高景輝	8
倪蕙芳	387
張淑貞	218
曹進義	44, 94, 247, 401
曹麗玉	347
梁世祥	218
粘君琪	15, 346
莊曜駿	292
許北辰	224, 283
許淼淼	258
連怡鈞	28
郭介煒	28
郭素真	28
郭鴻裕	365
陳君弢	191, 301
陳怡如	181, 191, 336
陳泊菘	309
陳金枝	191, 229, 308
陳柱中	365
陳威臣	44, 94, 247, 401
陳柏亨	209
陳柏宏	166
陳祈男	166
陳涵葳	209
許健輝	365
馮騰永	209
黃巧雯	387

黃守宏 166
黃春惠 229
黃哲倫 73
黃晉興 309
黃淑苓 16
黃毓斌 15, 346
黃瓊慧 166
楊正釧 292
楊宏仁 387
楊佐琦 209
楊崇民 15, 346
楊淑惠 355
楊惠婷 355
董耀仁 224, 283
廖大經 28, 54, 82
蔡志濃 318
蔡佳欣 16
蔡惠玲 318
蔡錦慧 191, 270, 308
鄧汀欽 191, 270, 308
鄭允 15, 346
鄭佳怡 365
蔣淑琦 365
劉滄棽 367
盧虎生 292
賴永昌 73
賴信順 181
賴素玉 387
謝靜敏 292
簡伊萱 191
羅淑芳 73
羅惠齡 258
關政平 209
蘇俊峰 309

JOURNAL OF TAIWAN AGRICULTURAL RESEARCH

(Formerly Journal of Agricultural Research of China)

Authors Index

Ann, Pao-Jen 27, 322
Chang, Shu-Chen 223
Chen, Chi-Nan 180
Chen, Chin-Chih 208, 246, 301
Chen, Chu-Chung 376
Chen, Chun-Tao 191, 301
Chen, Han-Wei 217
Chen, Po-Heng 217
Chen, Po-Hung 180
Chen, Po-Sung 317
Chen, Uei-Chern 53, 105, 257, 413
Chen, Yi-Ju 190, 208, 323
Cheng, Chia-Yi 376
Cheng, Edward Yun 14, 337
Chiang, Fen-Lang 246
Chiang, Ming-Yaw 14, 337
Chiang, Shu-Chi 364
Chien, Yi-Hsuan 208
Chiu, Yi-Chung 190
Chou, Chien-Ming 208, 301
Chou, Szu-Yi 93
Chuang, Yao-Chun 300
Deng, Ting-Chin 208, 282, 301
Dong, Yaw-Jen 228, 291
Feng, Teng-Yung 217
Guo, Horng-Yuh 376
Ho, Chyi-Chen 223
Ho, Ya-Ting 376
Hsia, Chi-Ni 53, 105, 257, 413
Hsieh, Ching-Ming 300
Hsiu, Bei-Chen 228, 291
Hsu, Kuang-Ping 300
Hsu, Miao-Miao 269
Huang, Che-Lun 81
Huang, Chiao-Wen 400
Huang, Chiung-Hui 180
Huang, Chun-Huey 246
Huang, Jin-Hsing 317
Huang, Shou-Horng 180
Huang, Yu-Bing 14, 337
Hung, Ting-Hsuan 27
Hwang, Shu-Ling 27
Kao, Ching Huei 1
Kuan, Cheng-Ping 217
Kuo, Chieh-Wei 43
Kuo, Su-Chen 43
Lai, Hsin-Shun 190
Lai, Su-Yu 400
Lai, Yung-Chang 81
Lee, Chi-Feng 107
Lee, Hsiang-I 269
Lee, Shough-Peng 269
Lee, Ya-Lin 300
Liang, Shih-Hsiang 223
Liao, Dah-Jing 43, 72, 93
Lien, I-Chun 43
Lin, Ching-Yi 386
Lin, Chu-Ping 322
Lin, Feng-Chyi 190, 208, 323
Lin, Hui-Ju 386
Lin, Mei-Ju 282
Lin, Yann-Rong 43

Lin, Yi-Ting 282
Lin, Yu-Wen 376
Liu, Tsang-Sen 376
Lo, Hui-Ling 269
Lo, Shu-Fang 81
Lu, Hsiu-Ying 14, 337
Lu, Yun-Sheng 27
Lur, Huu-Sheng 300
Ni, Hui-Fang 400
Nien, Chun-Chi 14, 337
Sekerka, Lukáš 107
Su, Jiunn-Feng 317
Syu, Chien-Hui 376
Triapitsyn, Serguei V. 113
Tsai, Chia-Hsin 27
Tsai, Chin-Hui 208, 282, 301
Tsai, Hui-Ling 322
Tsai, Jyh-Nong 322
Tsao, Chin-Yi 105, 257, 413
Tsao, Jhin-Yi 53
Tsao, Li-Yu 354
Wang, Chin-Ling 323
Wang, Shun-Cheng 223
Wang, Tai-Chuan 180
Wang, Zu-Hsien 14, 337
Wu, Chao-Jung 400
Wu, Dong-Hong 14, 72, 337
Wu, Pei-Chen 72
Wu, Rung-Yi 257
Wu, Tzu-Ying 53, 105, 257, 413
Wu, Yong-Pei 43
Xu, Min-QI 81
Yang, Chung-Min 14, 337
Yang, Hong-Ren 400
Yang, Hui-Ting 364
Yang, Jeng-Chuann 300
Yang, Shu-Hui 364
Yang, Tso-Chi 217

台 灣 農 業 研 究

關鍵詞索引

Ganoderma boninense	318
Pseudomonas mediterranea	16
RT-PCR檢測	229
土壤清洗	365
大量繁殖	401
小果油茶籽	292
小盾板前剛毛	15
小黃瓜	224
小黃薊馬	181
已酸	224
中胸背板剛毛	346
丹參	94
丹參酮	94
分子輔助回交育種	54
分子標幟輔助選拔	28
分蔥潛隱病毒	270
分類	336
分類學	112
巴拿馬病	209
毛狀根	94
水楊酸甲酯	283
水蜜桃	218
水稻	8, 28, 54, 82, 365
台灣	165, 336
玉米褪綠斑駁病毒	191
瓜實蠅	15, 346
甘蔗嵌紋病毒	191
甘藷	73
生命表	181
生物防治	283
生長抑制劑	44
禾本科	112
全長度核酸序列	229
名錄	165
多元抗體	229
多倍數	94
多原抗體	308
多效唑	44
朱頂紅	44
西方墨漬法	308
免疫體原	308
卵寄生蜂	165
抑芽劑 CIPC	73
抗病基因	54
亞磷酸	377
油脂品質	292
油茶籽儲藏條件	292
矽元素	8
空間分布	166
金花蟲	112
阿茲海默氏症	355
青枯病	377
品種篩選	258
柑橘	387
柑橘褐斑病	387
柑橘褐斑病菌	387
柑橘銹蟎	166
流式細胞儀	247
秋水仙素	247
耐旱	28
耐病性	209
耐鹽	28
苯甲基腺嘌呤	401
香菇柄水萃物	355

香蕉	209
香蕉黃葉病	209
倍體數	247
原球體	247
宮燈百合	229
捕食性天敵	218, 283
狹長孢靈芝	318
病毒	308
病害調查	191
胰島素	355
茭白	309
馬鈴薯	377
寄主植物	112
巢式-聚合酶鏈鎖反應	270
捲葉	112
族群動態	166
液-固態培養	44, 403
現地試驗	365
產期調控	309
棉蚜	224
氯化鐵	365
番茄	16
番椒微斑病毒	347
發育速率	181
貯藏	73
週年生產	309
新種	165
溫度	181
葉蟎	218
鈷 60 照射	73
嘉蘭條斑嵌紋病毒	229
種子含水率	292
種子消毒	347
管薊馬科	336
誘導防禦	224
寬薊馬亞科	336
數量遺傳	15, 346
稻熱病	54, 82
稻熱病病圃	82
蔥科作物	270
蔬菜栽培	258
蝴蝶蘭	247
遮蔽率	258
器械消毒	347
糖尿病	355
親緣分析	191, 270
鞘蛋白	308
儲藏溫度	292
檢測試劑	191
營農型光電溫室	258
舉臂式十字迷宮	355
薑	401
隱性基因	15
檬果	181
檳榔	318
鎘	365
鎘逆境	8
離體培養	94
藥劑篩選	16, 387
纓小蜂科	165
纓翅目	336
髓壞疽病	16

JOURNAL OF TAIWAN AGRICULTURAL RESEARCH

(Formerly Journal of Agricultural Research of China)

Key Words Index

6-benzylaminopurine	413
Agrochemical screening	27
Allium species	282
Alternaria alternata	400
Alternaria brown spot	400
Alzheimer’s disease	364
Amaryllis	53
Bacterial wilt	386
Bactrocera (*Zeugodacus*) *cucurbitae*	9
Banana	217
Betel palm	322
Biological Control	291
Blast	93
Blast disease	72
Cadmium	376
Cadmium stress	1
Camellia brevistyla seed	300
Christmas bells (*Sandersonia aurantiaca* Hook)	246
CIPC	81
Citrus (*Citrus reticulata*)	400
Citrus rust mite	180
Co60 exposure	81
Coat portion	301
Colchicine	257
Cotton aphid	228
Coverage	269
Cucumber	228
Cultivar selection	269
Detection reagent	208
Developmental rate	190
Diabetes mellitus	366
Disease nursery	93
Disease resistant gene	72
Disinfection of cutting tools	356
Drought tolerance	43
Egg parasitoid	113
Elevated plus-maze	364
Ferric chloride	376
Field survey	208
Flow cytometry	257
Full-length sequence	246
Fungicides screening	400
Fusarium wilt	217
Ganoderma boninense	322
Gloriosa stripe mosaic virus (GSMV)	246
Hairy root	105
Harvest period regulation	317
Hexanoic acid	228
Host plant	107
Idolothripinae	323
Immunogen	301
In vitro culture	105
Induced resistance	228
Insulin	364
Leaf beetle	107
Leaf-roll	107
Lentinus edodes stipe extracts	366
Life table	190
Liquid-solid culture	53, 415
List	113
Maize chlorotic mottle virus	208

Mango 190
Marker assisted backcross 72
Marker-assisted selection 43
Mass propagation 413
Melon fly 337
Methyl Salicylate 291
Moisture content of seed 300
Mono-phenotype surge 9
Mymaridae 113
Nested-polymerase chain reaction 282
New species 113
Oil quality 300
Oilseed storage condition 300
On-site trial 376
Oryza sativa L. 72
Paddy rice 376
Panama disease 217
Peach 223
Pepper mild mottle virus 354
Phalaenopsis 257
Phlaeothripidae 323
Phosphoric acid 386
Photovoltaic greenhouse 269
Phylogenetic analysis 208, 282
Pith necrosis 27
Plant growth retardant 53
Poaceae 107
Polyclonal antibody 246
Polyclonal antiserum 301
Polyploidy 105, 257
Population dynamic 180
Potato 386
PP333 53
Predator 223
Predators 291
Prescutellar bristle 337
Prescutellar bristles 9
Protocorm 257
Pseudomonas medietrranea 27
Quantitative genetic 337
Quantitative genetics 9
Rice 1, 43, 93
RT-PCR detection 246
Salt tolerance 43
Salvia miltiorrhiza Bunge 105
Scirtothrips dorsalis 190
Seed disinfection 354
Shallot latent virus 282
Silicon 1
Soil washing 376
Spatial distribution 180
Spider mite 223
Sprout inhibitor 81
Storage 81
Storage temperature 300
Sugarcane mosaic virus 208
Sweet potato 81
Taiwan 113, 323
Tanshinone 105
Taxonomy 107, 323
Temperature 190
Thysanoptera 323
Tolerance 217
Tomato 27
Vegetable cultivation 269
Virus 301
Water bamboo 317
Western blot 301
Year round harvest 317
Zingiber officinale 413

台灣農業研究　評閱者名單

第 67 卷 (2018)

尤進欽
國立宜蘭大學園藝學系
王強生
國立中興大學農藝學系
王智立
國立中興大學植物病理學系
王毓華
農業試驗所農場管理組
古新梅
國立中興大學農藝學系
石憲宗
農業試驗所應用動物組
朱燕華
食品工業發展研究所產品及製程研發中心
吳文哲
國立台灣大學昆蟲學系暨研究所
宋妤
國立中興大學園藝學系
李奇峰
農業試驗所應用動物組
李昆達
國立台灣大學生化科技學系
杜宜殷
國立台灣大學園藝暨景觀學系
林仲平
國立台灣師範大學生命科學系
林宗俊
農業試驗所植物病理組
林宜賢
國立屏東科技大學植物醫學系
林彥蓉
國立台灣大學農藝學系暨研究所
林雅芬
國立台灣大學農藝學系暨研究所
柯俊成
國立台灣大學昆蟲學系暨研究所
洪挺軒
國立台灣大學植物病理與微生物學系
胡仲祺
國立中興大學生物科技學研究所
徐善德
國立嘉義大學園藝學系暨研究所
張念台
國立屏東科技大學植物醫學系
張俊哲
國立台灣大學昆蟲學系暨研究所
張清安
朝陽科技大學應用化學系
張雅君
國立台灣大學植物病理與微生物學系
莊益源
國立中興大學昆蟲學系
許正一
國立台灣大學農業化學系
許奕婷
國立中興大學農藝學系
郭美華
國立中興大學昆蟲學系
陳文華
國立屏東科技大學植物醫學系
陳宗祺
亞洲大學生物科技學系
陳金枝
農業試驗所植物病理組
陳凱儀
國立台灣大學農藝學系暨研究所
陳榮坤
台南區農業改良場
曾顯雄
國立台灣大學植物病理與微生物學系
黃文理
國立嘉義大學農藝學系
黃鵬林
國立台灣大學園藝暨景觀學系
楊正澤
國立中興大學昆蟲學系
楊曼妙
國立中興大學昆蟲學系
楊雯如
國立台灣大學園藝暨景觀學系
溫宏治
國立台灣大學植物科學研究所
葉文斌
國立中興大學昆蟲學系
葉淑丹
國立中央大學生命科學系
劉麗雲
實踐大學食品營養與保健生技學系
蔡文錫
國立嘉義大學植物醫學系暨研究所
蔡志濃
農業試驗所植物病理組
鄭明倫
自然科學博物館動物組
鄭秋萍
國立台灣大學植物科學研究所
蕭文鳳
國立嘉義大學植物醫學系暨研究所
蕭旭峰
國立台灣大學昆蟲學系暨研究所
賴鴻裕
國立中興大學土壤環境科學系
戴淑美
國立中興大學昆蟲學系
謝廷芳
農業試驗所花卉研究中心
顏國欽
國立中興大學食品暨應用生物科技學系
羅筱鳳
國立台灣大學園藝暨景觀學系
蘇正德
東海大學食品科學系

Reviewers, Journal of Taiwan Agricultural Research

Volume 67, 2018

Chang, Chun-che
Department & Graduate Institute of Entomology, National Taiwan University

Chang, Niann-Tai
Department of Plant Medicine, National Pingtung University of Science and Technology

Chang, Chin-An
Department of Applied Chemistry, Chaoyang University of Technology

Chang,Ya-Chun
Department of Plant Pathology and Microbiology, National Taiwan University

Chen, Chin-Chih
Division of Plant Pathology, Taiwan Agricultural Research Institute

Chen, Kai-Yi
Department of Agronomy, National Taiwan University

Chen, Rong-Kuen
Tainan District Agricultural Research and Extension Station, COA

Chen, Tsung-Chi
Department of Biological Science and Technology, Asia University

Chen, Wen-Hua
Department of Plant Medicine, National Pingtung University of Science and Technology

Cheng, Chiu-Ping
Institute of Plant Biology, National Taiwan University

Chu, Yan-Hwa
Product and Process Research Center, Food Industry Reaserch and Development Institute

Chuang, Yi-Yuan
Department of Entomology, National Chung Hsing University

Dai, Shu-Mei
Department of Entomology, National Chung Hsing University

Do, Yi-Yin
Department of Horticulture and Landscape Architecture, National Taiwan University

Hseu, Zeng-Yei
Department of Agricultural Chemistry, National Taiwan University

Hsiao, Wen-Feng
Department of Plant Medicine, National Chiayi University

Hsieh, Ting-Fang
Floriculture Research Center, Taiwan Agricultural Research Institute

Hsu, Yi Ting
Department of Agronomy, National Chung Hsing University

Hsu, Shan-Te
Department of Horticulture, National Chiayi University

Hu, Chung-Chi
Graduate Institute of Biotechnology, National Chung Hsing University

Huang, Pung- Ling
Department of Horticulture and Landscape Architecture, National Taiwan University

Huang, Wen-Li
Department of Agronomy, National Chiayi University

Hung, Ting-Hsuan
Department of Plant Pathology and Microbiology, National Taiwan University

Jeng, Ming-luen
National Museum of Natural Science

Ko, Chiun-Cheng
Department & Graduate Institute of Entomology, National Taiwan University

Ku, Hsin-Mei
Department of Agronomy, National Chung Hsing University

Kuo, Mei-Hwa
Department of Entomology, National Chung Hsing University

Lai, Hung-Yu
Department of Soil and Environmental Science, National Chung Hsing University

Lee, Chi-Feng
Division of Applied Zoology, Taiwan Agricultural Research Institute

Lee, Kung-Ta
Department of Biochemical Science & Technology, National Taiwan University

Lin, Chung-Ping
School of Life Science, National Taiwan Normal University

Lin, Ya-Fen
Department of Agronomy, National Chung Hsing University

Lin, Yann-Rong
Department of Agronomy, National Taiwan University

Lin, Tsung-Chun
Division of Plant Pathology, Taiwan Agricultural Research Institute

Lin,Yi-Hsien
Department of Plant Medicine, National Pingtung University of Science and Technology

Liu, Li-Yun
Department of Food Science, Nutrition, and Nutraceutical Biotechnology, Shih Chien University

Lo, Hsiao-Feng
Department of Horticulture and Landscape Architecture, National Taiwan University

Shiao, Shiuh-Feng
Department & Graduate Institute of Entomology, National Taiwan University

Shih, Hsien-Tzung
Division of Applied Zoology, Taiwan Agricultural Research Institute

Su, Jeng-De
Department of Food Science, Tunghai University

Sung, Yu
Department of Horticulture, National Chung HsingUniversity

Tsai, Jyh-Nong
Division of Plant Pathology, Taiwan Agricultural Research Institute

Tsai, Wen-Shi
Department of Plant Medicine, National Chiayi University

Tzean, Shean-Shong
Department of Plant Pathology and Microbiology, National Taiwan University

Wang, Chan-Sen
Department of Agronomy, National Chung Hsing University

Wang, Chih-Li
Department of Plant Pathology, National Chung Hsing University

Wang, Yu-Hua
Division of Farm Management, Taiwan Agricultural Research Institute

Wen, Hung-Chich
Institute of Plant Biology, National Taiwan University

Wu, Wen-Jer
Department & Graduate Institute of Entomology, National Taiwan University

Yang, Jeng-Tze
Department of Entomology, National Chung Hsing University

Yang, Man-Miao
Department of Entomology, National Chung Hsing University

Yang,Wen-Ju
Department of Horticulture and Landscape Architecture, National Taiwan University

Yeh, Shu-Dan
Department of Life Science, National Central University

Yeh, Wen-Bin
Department of Entomology, National Chung Hsing University

Yen, Gow-Chin
Department of Food Science and Biotechnology, National Chung Hsing University

Yiu, Jinn-Chin
Department of Horticufture, National Ilan University

「台灣農業研究」稿約

一、 本刊為行政院農業委員會農業試驗所發行之學術性期刊 (季刊)，刊登國內、外農業試驗研究相關之研究稿件：

專題論述：由本所期刊編審會或總編輯邀請專家撰寫。

研究報告：關於農業科學未曾發表之研究報告，按題目、作者、摘要、前言、材料與方法、結果、討論、結論 (一般略去，或併入討論)、誌謝 (可略去)、引用文獻等依序撰寫，表與圖附於其後，全文以不超過10印刷頁為原則。其中，'結果' 與 '討論' 如有必要，可合併成'結果與討論'。

研究簡報：關於農業科學未曾發表之簡短原始研究報告，不須分章節撰寫 (亦可參照研究報告次序)，但須附中英文摘要，全文以不超過4印刷頁為原則。

二、 稿件應以中文或英文撰寫，以文書軟體MS WORD®格式儲存成檔案 (檔案類型以Word 97-2003或以上版本文件為佳)。文稿以A4大小 (長29.6公分，寬21公分) 兩倍行高 (double space) 繕打，加行號，12號字體，左邊對齊，版面應預留上下3公分，左右2公分之空間，並自第二頁起在右下角以阿拉伯數字註明頁數。撰寫時，中文以細明體、英文以Times New Roman字體打字；英文字距及句距皆一字節。

三、 投稿者在本刊線上投審系統首頁點選'投稿者區域'，在一般稿件提交區點選'作者提交新稿件'進入，確認及點選稿件已依照稿約撰寫無誤後，下一步即填寫稿件資料，務請填寫封面信函，再依序填寫作者資料、上傳檔案及確認提交。

四、 稿件格式：

題　　目：宜簡短，能充分反應文旨，並附簡題 (Running title) (中文12字；英文6字以內為原則)。英文題目除介系詞、連接詞等字外，每字第一字母應大寫。報告如有機關所屬編號，請列於誌謝。

作　　者：作者姓名置中，作者二人或二人以上時，以中文一全形空格間隔之；英文姓名則名在前姓氏在後，作者二人或二人以上時，以 " , " 相隔，最後一人前加 "and" 字樣，其表示方法如Chen-Seng Huang, Chen-Chang Chen, and Chyr-Guan Chen。每一作者須附小註，以說明所屬機關單位與職稱，通訊作者附予星號" * "，並須附電子郵件信箱。題目與作者同置第一頁。

摘　　要：不分段落、宜簡潔明確。摘要之前應加作者、年代、題目、期刊、及卷期，摘要之後應附關鍵詞 (Key words)，關鍵詞不超過五個，每個英文關鍵詞第一字母應大寫。摘要以不超過五百字為原則。以中 (英) 文發表者須附英 (中) 文摘要，置於本文之後，且內容須與中 (英) 文摘要相符，並附通訊作者、作者之小註。

本　　文：依序為前言 (INTRODUCTION)、材料與方法 (MATERIALS AND METHODS)、結果 (RESULTS)、討論 (DISCUSSION)、結論 (CONCLUSIONS)、誌謝 (ACKNOWLEDGMENTS)、引用文獻 (REFERENCES)。除學名、專有名詞、化學式、數學公式、數字、度量衡單位及縮寫外，文句中不得中外文並用，需使用英 (外) 文以助明瞭時，須將英 (外) 文括號置於中文之後。材料與方法、結果兩部分如有必要須分段 (節)，每一段內並可再分小段，以助明瞭文稿內容。各段題目 (heading) 單行置於該段之前，小段題目 (subheading) 直接置於小段之首。如因結果繁複不易聯接討論，可合併成'結果與討論 (RESULTS AND DISCUSSION)'。如有附錄，請置於引用文獻之前。

表　　格：

1. 每一表格應分別繕製，須以MS WORD®中 “表格” 工具製作，上方須有標題，以句點結尾，並按文中出現順序加上表**1. (Table 1.)**，表**2. (Table 2.)** 等冠號。
2. 中文稿之表格標題應中英文並列，冠號與表幅齊頭，表內項目及內容以英文書寫。英文標題及各欄英文細目，除第一字之第一字母大寫外，餘均小寫。單位使用SI units (如 $g\ L^{-1}$、$Mg\ ha^{-1}$、$m\ s^{-1}$)，一律單數、英文字母。
3. 表格應加分析、簡化與歸納後始可列表。每一表格應在文中標示清楚，以便對照，且每一表格應單頁製作。
4. 表中內容如需說明，均須附加小註，以z, y, x, ...等縮小小寫字母置於右上角，並於表下依次敘述。表格僅列橫線。星號 “* (5%顯著性水準)”、“** (1%顯著性水準)” 保留統計顯著性使用。
5. 性質類同之圖與表，務請精簡與合併。

圖　　片：

1. 手繪圖版以黑色墨水於白色紙上分別繪製，掃描後存成JPG電子檔 (或JPEG電子檔) 上傳。如以電腦繪圖，力求清晰，亦存成JPG檔 (或JPEG檔) 上傳，或直接貼於本文之後。請勿使用MS Office內建軟體繪圖，建議使用專業繪圖軟體 (如Sigma Plot) 繪製。
2. 圖中所有標示應以英文書寫 (Arial字型) 或註明於標題之後小標一律以(A), (B), (C), ...編號，分類學則可以1, 2, 3, ...標示。
3. 照片以光面紙為原則，清晰度以縮小50%可判讀為宜。多幅照片儘量製成併版；如有特別指明，應加圈或箭頭表明。顯微照相，應加比例尺大小；照片大小以不超過版面 (25 cm × 18 cm) 為宜，掃描後存成JPG檔 (或JPEG檔) 上傳，解析度300 dpi或以上，並直接貼於本文之後。
4. 圖版及照片應有標題，並按文中出現的順序加上 **(圖1) (Fig. 1)**、**(圖2) (Fig. 2)** 等冠號，在文中須標示清楚，以便對照。中文稿標題應中英文併列，英文稿標題以英文書寫即可，力求明確。
5. 除併圖外，每一圖片應單頁製作。
6. 圖版及照片未達印製之解析度要求，文稿將不予刊登。

誌　　謝：不分段落，包括人物、經費來源、報告之機關賦予編號等。誌謝可略去。

引用文獻：

1. 列舉確實曾引用之文獻。文獻以登載於學術性期刊、書籍、研討會之文章及學位論文為限 (不含推廣報告)，如引用網路學術性報告，須加註瀏覽、下載日期及網址路徑。
2. 文中涉及引用文獻時，根據該文獻的作者與年代加以括號：如 (Sung 1991)、(Lo 1991, 1992)、(Chen & Ho 1983)、(Sung 1991; Hsu *et al*. 1992)，以年代先後為序置於引用文處。
3. 文獻一律以英文撰寫，以姓名字母為序。作者相同時以年代先後為序。
4. 文獻之書寫方法以期刊為例，依序為作者、年代、題目、期刊、卷數、頁數。在每卷每期之頁碼都從數字 1開始的期刊，才必須加上期數。
5. 期刊名須依該期刊公告之縮寫撰寫；如無者，請參照Biosciences Information Service (1992) 出版之 “Serial Sources for the BIOSIS Previews Database” 或依該學術領域之慣例撰寫。

6. 文獻書寫範例如下：

(1) 原中文報告引用文獻出自學術性期刊

Hsu, S. H., T. Y. Chung, T. C. Kuo, and S. F. Wang. 1992. Studies on the micro-climate of greenhouse. I. Light environment of greenhouse. J. Agric. China 157:34–43. (in Chinese with English abstract)

(2) 原中文報告引用文獻出自著作性書籍

Sung, J. M. 1991. Nitrogen Metabolism of Rice. Chinese Agronomy Press. Taipei. 100 pp. (in Chinese)

(3) 原中文報告引用文獻出自編輯性書籍

Yang, C. M. and C. Y. Lin. 2003. Studies on rice precision farming system. p.1–12. *in*: Rice Precision Farming System. (Yang, C. M. and C. Y. Lin, eds.) Taiwan Agric. Res. Inst. Pub. No. 105. Taichung, Taiwan. 252 pp. (in Chinese with English abstract)

(4) 原中文報告引用文獻出自研討會專刊

Chang, C. A. 2001. Application and extension of virus-free seedling for the control of virus diseases of passion fruit in Taiwan. p.31–38. *in*: Proceeding of Symposium on Application of Healthy Plant for Control of Plant Diseases. November 24, 2001. Taichung, Taiwan.Taiwan Phytopathol. Soc. Publ., Taichung. (in Chinese with English abstract)

(5) 原報告引用文獻出自特定網頁資料

Lehtomäki, A. 2006. Biogas production from energy crops and crop residues. Jyväskylä Stud. Biol. Environ. Sci. 163. Univ. of Jyväskylä, Jyväskylä, Finland. https://jyx.jyu.fi/dspace/bitstream/handle/123456789/13152/9513925595.pdf (visit on 12/16/2014)

五、 單位、縮寫格式、括號及統計分析

1.凡文中出現之數值，除非另有特別之意義，一律用阿拉伯數字書寫。數字在句首之英文稿，改以文字表示。單位則用國際公認標準符號 (SI units) 代替全文，各常用之縮寫單位參照說明如下：

(1) 長度：公里 (km)、公尺 (= 米) (m)、公分 (= 厘米) (cm)、公釐 (= 毫米) (mm)、微米 (μm)。

(2) 重量 (W)：公噸 (Mg)、公斤 (kg)、公克 (g)、毫克 (mg)、微克 (μg)。

(3) 容積 (V)：公升 (L)、公撮 (= 毫升) (mL)、微升 (μL)。

(4) 面積：公頃 (ha)、平方公尺 (m^2)、平方公分 (cm^2)。

(5) 溫度：攝氏 (℃)、絕對溫度 (K，但須加以說明)。

(6) 時間：日 (天) (d)、時 (h)、分 (min)、秒 (s)。

(7) 濃度：莫耳 (M)、千分之一莫耳 (mM)、當量 (N)、千分之一當量 (mN)。

(8) 重力加速度：*g*。

(9) 分子量：kDa。

(10) 肥料三要素：如以肥料種類$N\text{-}P_2O_5\text{-}K_2O$，如以元素N-P-K。

2.其他之縮寫：平均 (*avg*.)、大約 (*ca*.)、直徑 (diam.)。

3.括號：括號字型全以半形 ( )，括號前後各空一格。

4.統計分析：達顯著性水準以*、**或處理間顯著差異區分，以a、b、c、…表示者均應加註顯著機率值*P* (例如$P < 0.01$)；各統計量請採慣用符號，例如相關係數 (*r*) 、決定係數 (R^2)、變異係數 (cv)、標準差 (sd)、平均值標準差 (se) 等。

5.其他問題：

(1) 單位與其前面數值之間應空一格，如220 g。

(2) 數學運算符號與其前後數值之間應各空一格，如$P = 0.014$、23.6 ± 3.0、15×2。

(3) 在 ±、× 或to的前後兩數值若是含有單位，請用23.6% ± 3.0%、15 cm × 2 cm、20℃ to 30℃ (即勿省略前面數值之單位)。

(4) 數值起迄符號用– (半型破折號；EN Dash)，勿用 ~ 或 -，且與前後兩數值之間無須留空格，如3–6、2005–2007年、7月20–25日。

(5) 年代一律用西元。

(6) 濃度與容量單位使用mg L^{-1} (勿用ppm)。

(7) 轉速單位使用× *g* (勿用rpm)。

六、 其他注意事項

1. 本刊採網路線上投審，來稿請至本刊線上投審系統網址 (http://aspers.airiti.com/JTAR) 進行投稿作業，投稿前務請詳閱稿約及投稿須知，並可參閱最新一期報告。
2. 生物學名以斜體字表示，屬名第一字母大寫。學名於摘要、本文、每一圖表中第一次出現時，須寫全名，如*Citrus sinensis*；第二次出現時，屬名保留第一字母後加句點 “.”，種名全寫，如*C. sinensis*。生物學名於本文中首次出現時應加註命名者，如*Oryza sativa* L.。
3. 中文稿件中，外文名詞應儘量翻譯成中文，若是不常見名詞應於文中第一次出現時以括號標出原文。摘要及本文中，英文縮寫於第一次出現時，須附全名。圖版及照片如有英文縮寫，應於標題中附全名，表格內之英文縮寫，須於小註附全名。
4. 農作物品種名須加上單引號，中文用「 」(如水稻「台稉9號」、木瓜「台農2號」)，英文用‘’ (如rice ‘TK9’, papaya ‘TNG2’)。

七、 投稿方式

1. 稿件內容務須依照上列規定格式書寫及線上投稿，本期刊編審會保有修改與退稿之權利。文稿經本期刊接受後，由作者負責前二校。除筆誤與錯字外，作者在校稿時，不得擅自更改內容與數據。
2. 投稿前請詳閱稿約及參見最一期刊登報告，投審稿件程序請上網參閱本期刊網址：http://aspers.airiti.com/JTAR。

八、 投稿本期刊之稿件，不得投稿其他刊物。稿件經本期刊接受後，刊登權屬發行單位農業試驗所所有，非經本所書面同意，不得轉載或移轉他處發表。本期刊具專屬版權，恕不受理一稿兩投之文章 (已刊載或投寄國內外有版權刊物之文章，請勿再投稿本刊)。若有上述情事除依相關法律處理責由作者自負外，本刊將列入紀錄永不受理其投稿。

九、 本刊以黑白印製為原則，彩圖經編審會同意得以彩色編印。本期刊彩圖編印收費標準調整如下表，由作者自行向廠商支付，未付清前稿件將不予刊登。

彩圖編印收費標準：

總張數	總價 (元)	計價公式
1	5,000	5,000 × 1
2	7,000	5,000 × 1 + 2,000 × 1
3	9,000	5,000 × 1 + 2,000 × 2
4	11,000	5,000 × 1 + 2,000 × 3
5	13,000	5,000 × 1 + 2,000 × 4
6 張以上	14,500 起	5,000 × 1 + 2,000 × 4 + 1,500 × (n – 5)

十、 其他未盡事宜本期刊得隨時修正或補充。

Instructions to Authors

Journal of Taiwan Agricultural Research (revised on 1 November 2016)

General Information

The *Journal of Taiwan Agricultural Research* (JTAR) is published four issues annually by the Taiwan Agricultural Research Institute (TARI). Researchers and scientists willing to make the contribution in the fields of agricultural development by publishing their full papers of original research, reviews, or short communications in the Journal are highly welcome and appreciated. Research staff affiliated with TARI is not a requirement for publishing in JTAR. All manuscripts should be submitted online at the Journal's website, http://aspers.airiti.com/JTAR. Follow its instructions. Submissions to JTAR must not simultaneously submit to any other scientific or technical journal.

Scope

After critical review and approval by the editorial board, JTAR publishes articles in plant science; biotechnology; agricultural chemistry; plant protection; agricultural engineering; agricultural economics and extension; industrial strategic management; biostatistics; and bioinformatics. Articles reporting experimentation or research findings in these fields will be peer-reviewed as research articles. Short articles (no more than 4 printed pages) concerned with experimental techniques, results, or unique phenomena will be peer-reviewed as short communications. Review articles will be under invitation by the editorial board of JTAR.

Style

See the latest issue of JTAR for style and layout. General rules are as follows:

- All abbreviations should be defined at the first mention and in tables and figures, and be used throughout the entire article.
- The Latin names of organisms (e.g., plants, pathogens, insects and pests) must always list when appeared at first appearance (in Abstract or text). Give the complete binomial and authorities.
- At first listing both the common and chemical names of pesticides must be given.
- SI units must be used in the manuscript.
- Use the 24-h time system (e.g., 0200 and 1400) and abbreviate dates such as 3 October 2015 or 10/03/2015.
- Except when used with units, spell out numbers one through nine. Put commas for the decimal separator (e.g., 1,356,078 and 6,078) and place a zero before the decimal point (e.g., 0.6078 and 0.078).

Preparation of Manuscript Files

All submitted manuscript files should write in Chinese or English and save it in Microsoft Word 97-2003 or later versions (.doc files or .docx files). Other word processing software is not accepted. High-resolution figure files may be included in manuscript and submitted separately in forms of .jpg files

or .doc files or .docx files, one figure per file. Do not use word processing features such as automated bulleting and numbering, head and subhead formatting, or internal linking. Text should be typed in A4 size (29.6 cm long, 21 cm wide) in double-spaced and be numbered and aligned to the left side, while tables use single-spaced. Only one font and size (font no. 12) is used. Provide the document with an upper and lower margin of 3 cm and a left and right margin of 2 cm. Starting from the second page, indicate the page number on the lower right corner. When typing the manuscript, use the font of MingLiu (細明體) for Chinese and Times New Roman for English. Leave a single space between each English word and sentence. The author submitting the manuscript should click on "Authors" on the front page of Journal's Online Submission and Review System. Under "Author" page, click "Submit a new manuscript". Confirm submission checklist and then complete the information related to the manuscript including a cover letter.

Follow the sequence and instructions to fill out and upload the information. JTAR adopts a double-blind review for all submitted manuscripts. Reviewers will be able to download the submitted files that authors upload to the system. There are three main types of articles published by JTAR, i.e., "Reviews", "Research Articles", and "Short Communications".

Reviews. A review article is invited by editor-in-chief of JTAR and will be reviewed and revised to assure an appropriate style and content.

Research Articles. Write a research article according to the mentioned scope in the following sequence: title, author(s), abstract, and text. Attach tables, figure captions, and figures at the end of text. In general, each manuscript should not exceed 10 printed pages.

Short Communications. An article that has a size less than 4 printed pages concerned with experimental techniques, results, or unique phenomena may be published as a short communication when accepted.

Title. A title highlights the subject and purpose of the article briefly but clearly. Use common names of crops, avoid abbreviations, and limit words where possible. Except prepositions and connectives, capitalize each word in the English title. Write down the suggested running title, with no more than twelve Chinese characters or six English words, in the next sentence.

Author. List names of all authors below the title and place an asterisk after the corresponding author. The next sentence lists professional titles, institutions and complete addresses of all authors. Provide e-mail address of the corresponding author. Place title and author(s) on the same page.

Abstract. Each article has a single self-contained paragraph, containing the rationale, objectives, methods, results, and their meaning or scope of application specifically. Identify the important details to interpret the results but try not to exceed five hundred words. Do not cite references and avoid equations. In the next sentence, include no more than five key words and capitalize the first letter of each word. If the manuscript written in Chinese (English), place English (Chinese) abstract after the main text of the manuscript.

Text. The text includes the following sections in sequence: INTRODUCTION, MATERIALS AND METHODS, RESULTS, DISCUSSION, CONCLUSIONS (optional), ACKNOWLEDGMENTS (may be omitted), and REFERENCES. If required, combine the results and discussion section into "RESULTS AND DISCUSSION", and incorporate the conclusions into the discussion. If there is an appendix, place

it before the references. Except necessary, do not mix English with other languages. If required, place headings and subheadings at the head of paragraphs of the sections MATERIALS AND METHODS and RESULTS.

Tables. Prepare tables with the tables feature of MS WORD®; do not use tab keys, space bars, or graphics boxes. Number tables consecutively with one table per page. Place tables at the end of text. Each datum needs to be contained in a defined cell. Table heads should be complete and self-contained but brief. All variables should be defined and abbreviations be spelled out in table footnotes. Follow the order of z, y, x, w, v, u, …. The *, **, and *** are used only to show statistical significance at the 0.05, 0.01, and 0.001 probability levels, respectively. Lack of significance indicates as NS. Asterisks or letters next to values indicate statistical significance and should align them in the same cell as the value. All footnotes should be set on separate lines. Capitalize only the first word in a row of the body of a table with the exception of proper nouns. Use only SI units (e.g., $\mu g\ L^{-1}$, $Mg\ ha^{-1}$, and $m\ s^{-1}$) in the singular forms.

Figures. Provide clear contrast figures in high-resolution JPG files (≧ 300 dpi) and upload them on-line as individual files and attach them after tables; one figure one page. Keep relative sizes of symbols, letters, and numbers as the original should be larger than the intended final size. Use English for letters in Arial font. Explain curves or symbols in a legend within the figure. JTAR will publish figures in black and white. Color figures, images, photos or maps are subject to the agreement of the editorial board and fee must be paid before the manuscript is published in the journal. Photographs and drawings for graphs and charts should be prepared with good contrast of dark and light and no more than the size of 25 cm × 18 cm. Use English for figure captions and separate captions for figures. A caption should be concise but given in sufficient detail. Define the crop or soil involved, the major variables presented, the place and year, and the abbreviations and symbols used in the caption. Indicate the scale for illustrations.

Acknowledgments. If required, provide it in a single paragraph. List institutional sponsors, the institutional article number, and contribution acknowledgment.

References. The reference section lists published literature and unpublished reports, theses, and dissertations cited in the manuscripts. Alphabetize the list by the surnames of the first author, followed by the other authors. Cite unpublished data, personal communications, and reports in the text only (in parentheses). Give the authors, year, complete article title, abbreviated journal title, volume number, and inclusive pages for journal articles. Give the authors, year, chapter title, pages, complete book title, book editor, publisher, and place of publication for book chapters. Add also the place and date of the conference for proceedings. For electronic references and others, see the current issues of JTAR. Single author references precede multiple-author references for the same first author. Use chronological order for references with identical authorship. Add a lowercase letter (i.e., a, b, c, etc.) to the year to identify same-year or multiple-author references for text citation. Write the author and year of the cited references inside parentheses; for example, (Chen & Ho 1983; Sung 1991; Hsu *et al.* 1992). Refer to the "Serial Sources for the BIOSIS Previews Database" published by Biosciences Information Service (1992) for the abbreviations of an academic journal. Some reference examples:

- Reference from an academic journal written in Chinese:
  Hsu, S. H., T. Y. Chung, T. C. Kuo, and S. F. Wang. 1992. Studies on the micro-climate of greenhouse. I. Light environment of greenhouse. J. Agric. Res. China 157:34–43. (in Chinese with English abstract)
- Reference from a book written in Chinese:
  Sung, J. M. 1991. Nitrogen Metabolism of Rice. Chinese Agronomy Press. Taipei. 100 pp. (in Chinese)
- Reference from an edited book written in Chinese:
  Yang, C. M. and C. Y. Lin. 2003. Studies on rice precision farming system. p.1–12. *in*: Rice Precision Farming System. (Yang, C. M. and C. Y. Lin, eds.) Taiwan Agric. Res. Inst. Pub. No. 105. Taichung, Taiwan. 252 pp. (in Chinese with English abstract)
- Reference from a proceedings written in Chinese:
  Chang, C. A. 2001. Application and extension of virus-free seedling for the control of virus diseases of passion fruit in Taiwan. p.31–38. *in*: Proceeding of Symposium on Application of Healthy Plant for Control of Plant Diseases. Taichung, Taiwan. November 24, 2001. Taiwan Phytopathol. Soc. Publ., Taichung. (in Chinese with English abstract)
- Reference from a website:
  Lehtomäki, A. 2006. Biogas production from energy crops and crop residues. Jyväskylä Stud. Biol. Environ. Sci. 163. Univ. of Jyväskylä, Jyväskylä, Finland. https://jyx.jyu.fi/dspace/bitstream/handle/123456789/13152/9513925595.pdf (visit on 12/16/2014)

Units, Abbreviations, and Statistical Analysis

Use the SI system (Systéme International de Unités) in JTAR. Some commonly used abbreviated units:

- Length: kilometers (km), meter (m), centimeter (cm), millimeter (mm), micrometer (μm)
- Weight (W): ton (Mg), kilogram (kg), gram (g), milligram (mg), microgram (μg)
- Volume (V): litre (L), millilitre (mL), microlitre (μL)
- Surface: hectare (ha), square meter (m^2)
- Temperature: Celsius (℃), absolute temperature (K, but must be indicated)
- Time: day (d), hour (h), minute (min), second (s)
- Concentration: mole (M), millimole (mM), normal (N), millinormal (mN)
- Gravitational acceleration: *g*
- Molecular weight: kDa
- Three macronutrients in fertilizers: element─N-P-K ; equaivalent quantity─$N\text{-}P_2O_5\text{-}K_2O$

  Other abbreviations:
- Average: *avg*.
- Approximate: *ca*.
- Diameter: diam.
- Concentration: e.g. mg L^{-1}.
- Frequency of a rotation: × *g*.

Statistical Analysis

Manuscripts should contain enough details of experimental design so that the results can be judged for validity and repeatability. The symbols * and ** are used to show differences in statistical significance. Add *P* value (e.g. $P < 0.01$) when a, b, c, ... are used to classify statistical significance. Examples of commonly used symbols: *r* for correlation coefficient, R^2 for coefficient of determination, cv for coefficient of variation, sd for standard deviation, and se for standard error.

Accepted Manuscripts

When a submitted manuscript is accepted, corresponding author will receive notification from an editor or editor-in-chief, and the accepted files will be used for press. Authors are responsible for obtaining all permissions for use of materials in the manuscripts from other publishers and should notify the editor at the time the manuscript is accepted. JTAR reserves the right to publish and republish any parts submitted with a manuscript.

Plagiarism

Manuscripts may be screened for plagiarism upon submission. If there appears significant duplicate content with other sources, the manuscript will be forwarded to the journal editor for evaluation and action.

Editorial Board

Editor-in-Chief

Yang, Chwen-Ming
Division of Crop Science, Taiwan Agricultural Research Institute

Editors

Ann, Pao-Jen
Division of Plant Pathology, Taiwan Agricultural Research Institute
Chang, Ruey-Jang
Division of Plant Pathology, Taiwan Agricultural Research Institute
Chang, Yung-Ho
Department of Food and Nutrition, Providence University
Chen, Chien-Chung
Division of Applied Zoology, Taiwan Agricultural Research Institute
Chen, Chiling
Division of Agricultural Chemistry, Taiwan Agricultural Research Institute
Chen, Chung-Li
Department of Agronomy, National Chung Hsing University
Chen, Iou-Zen
Department of Horticulture and Landscape Architecture, National Taiwan University
Chen, Suming
Department of Bio-Industrial Mechatronics Engineering, National Taiwan University
Chen, Yu-Hui
Department of Agricultural Economics, National Taiwan University
Chien, Li-Hsien
Department of Applied Economics, National Chung Hsing University
Chung, Wen-Hsin
Department of Plant Pathology, National Chung Hsing University
Do, Yi-Yin
Department of Horticulture and Landscape Architecture, National Taiwan University
Hou, Feng-Nan
Department of Entomology, National Chung Hsing University
Huang, Chao-Chia
Division of Crop Science, Taiwan Agricultural Research Institute
Huang, Jenn-Wen
Department of Plant Pathology, National Chung Hsing University
Huang, Pung-Ling
Department of Horticulture and Landscape Architecture, National Taiwan University
Huang, Yuh-Ming
Department of Soil and Environmental Sciences, National Chung Hsing University
Juang, Kai-Wei
Department of Agronomy, National Chiayi University
Kao, Ching-Huei
Department of Agronomy, National Taiwan University
Ko, Chiun-Cheng
Department & Graduate Institute of Entomology, National Taiwan University
Ku, Hsin-Mei
Department of Agronomy, National Chung Hsing University
Kuo, Bau-Jeng
Department of Agronomy, National Chung Hsing University
Lin, Huey-Ling
Department of Horticulture, National Chung Hsing University
Lin, Yann-Rong
Department of Agronomy, National Taiwan University
Liu, Li-Yu
Department of Agronomy, National Taiwan University
Lu, Hsiu-Ying
Miaoli District Agricultural Research and Extension Station
Lu, Kuang-Hui
Department of Entomology, National Chung Hsing University
Shen, Wei-Chiang
Department of Plant Pathology and Microbiology, National Taiwan University
Tsay, Jyh-Rong
Taiwan Agricultural Research Institute
Tseng, Menq-Jiau
Department of Horticulture, National Chung Hsing University
Tzou, Yu-Min
Department of Soil and Environmental Sciences, National Chung Hsing University
Wang, Chan-Sen
Department of Agronomy, National Chung Hsing University
Wang, Shu-Jen
Department of Agronomy, National Taiwan University
Wu, Wen-Jer
Department & Graduate Institute of Entomology, National Taiwan University
Yang, Jeng-Tze
Department of Entomology, National Chung Hsing University
Yen, Gow-Chin
Department of Food Science and Biotechnology, National Chung Hsing University

Editorial Assistants

Wang, Li-Jung
Editorial Office of Journal of Taiwan Agricultural Research
Wang, Zi-Yu
Editorial Office of Journal of Taiwan Agricultural Research
Wu, Huei-Ching
Division of Technical Service, Taiwan Agricultural Research Institute

Consultant to Editorial Office

Wu, Yi-Yan
Division of Technical Service, Taiwan Agricultural Research Institute

JOURNAL OF TAIWAN AGRICULTURAL RESEARCH

Vol. 67 No. 4 December 31, 2018

Published by Taiwan Agricultural Research Institute, Council of Agriculture

Publisher	Junne-Jih Chen
Editor-in-Chief	Chwen-Ming Yang
Address	No.189, Zhongzheng Rd., Wufeng Dist., Taichung City 41362, Taiwan, ROC
Website	http://www.tari.gov.tw; E-mail: jtar@tari.gov.tw; Tel: +886-4-2331-7236
Submission	http://www.ipress.tw/J0042
Press	Ainosco Press
Address	18F, No.80, Sec.1, Chenggong Rd., Yonghe Dist., New Taipei City 23452, Taiwan, ROC
Tel	+886-2-2926-6006
E-mail	press@airiti.com
Price	NT$ 175 (per copy)
Distributor	1. GB Books / 1F., No.209, Songjiang Rd., Taipei City 10485, Taiwan, ROC GB Website: http://www.govbooks.com.tw; Tel: +886-2-2518-0207 2. Wunan Books / No.6, Zhongshan Rd., Taichung City 40042, Taiwan, ROC Tel: +886-4-2226-0330